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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 5/06, 5/08, A61K 37/64
C07K 5/02

A1

(11) International Publication Number: WO 91/15507

(43) International Publication Date: 17 October 1991 (17.10.91)

(21) International Application Number: PCT/GB91/00538

(22) International Filing Date: 5 April 1991 (05.04.91)

(30) Priority data:
9008078.9 10 April 1990 (10.04.90) GB

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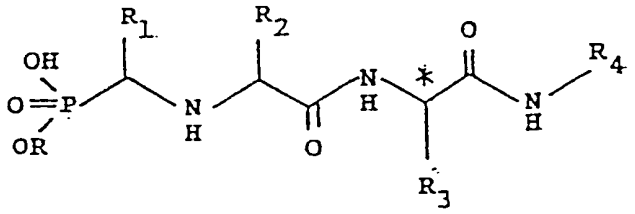
(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PHOSPHONOPEPTIDES WITH COLLAGENASE INHIBITING ACTIVITY



(I)

(57) Abstract

Phosphorous derivatives having structure (I), processes for their preparation and their use as collagenase inhibitors.

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PHOSPHONOPEPTIDES WITH COLLAGENASE INHIBITING ACTIVITY

The present invention relates to novel phosphorus derivatives, processes for their preparation and their use in medicine. In particular, the present invention relates to their use as inhibitors of enzymes of the collagenase family of neutral metalloproteases, for treating arthritic and other diseases.

The mammalian collagenase family of enzymes comprises a number of proteases, exemplified by interstitial (type I) collagenase itself, the stromelysins (also known as proteoglycanases or transins), fibroblast and polymorphonuclear leucocyte gelatinases (also known as collagen-IV-ases), and 'pump-1' (putative metalloprotease 1, uterine metalloprotease). Membership of the mammalian collagenase family of proteases is evident by possession of a number of highly characteristic and experimentally verifiable properties. [Goldberg et al., J. Biol. Chem. 2610, 6600, 1986; Whitham et al., Biochem. J. 240, 913, 1986; Breathnach et al., Nucleic Acids Res., 15, 1139, 1987; Muller et al., Biochem. J., 253, 187, 1988; Collier et al., J. Biol. Chem., 263, 6579, 1988; Murphy et al., Biochem. J., 258, 463, 1989; Quantin et al., Biochem. (N.Y.), 28, 5327, 1989; Birkedal-Hansen, J. Oral Pathol., 17, 445, 1988].

The range of therapeutic applications of the invention described hereinafter reflects the fundamental role of collagen and other proteinaceous substrates of the collagenase family of enzymes in the connective tissue matrix throughout the body. Applications extend to clinical interventions in many diseases and phenomena involving the destruction of collagen and other connective tissue components, and also normal or disordered tissue remodelling.

Inhibitors of the collagenase family of enzymes are considered to provide useful treatments for: arthritic diseases, such as rheumatoid and osteo-arthritis, soft tissue rheumatism, polychondritis and tendonitis; bone resorption diseases, such as osteoporosis, Paget's disease, hyperparathyroidism and cholesteatoma; the enhanced collagen destruction that occurs in association with diabetes; the recessive classes of dystrophic epidermolysis bullosa; periodontal disease and related consequences of gingival production of collagenase, or of PMNL collagenase release following cellular infiltration to inflamed gingiva, including by combating the greater susceptibility of diabetes patients to periodontal disease; corneal ulceration, e.g. that induced by alkali or other burns, by radiation, by vitamin E or retinoid deficiency; ulceration of the skin and gastro-intestinal tract, and abnormal wound healing; post-operative conditions, including colonic anastomosis, in which collagenase levels are raised; cancer, where members of the collagenase family of enzymes have been implicated in the neovascularization required to support tumour growth and survival [P. Basset et al., Nature, 348, 699, 1990] in the tissue remodelling required to accommodate the growing primary and secondary tumours, and in the penetration of tumour cells through the basement membrane of the vascular walls during metastasis; and demyelinating diseases of the central and peripheral nervous systems, including syndromes in which myelin loss is the primary pathological event and those in which demyelination follows axonal atrophy. The degradation of myelin in these diseases, exemplified by multiple sclerosis, is mediated by members of the collagenase family of enzymes.

As a particular example of the therapeutic value of inhibitors of the collagenase family of enzymes such as are disclosed in the present invention, chronic arthritic diseases leading to extensive loss of the collagen, proteoglycan and elastin components of the cartilage, bone and tendons within the joints, should be amenable to treatment with inhibitors of the collagenases, proteoglycanases (stromelysins) and gelatinases currently thought to be the major enzymes involved.

These enzymes have been detected in extracts of synovial and cartilage tissue, and have also been extensively studied in tissue cultures of a wide range of connective tissues. Apart from control of the biosynthesis, secretion and activation of the enzymes, the most important natural regulation of these enzymes in normal and diseased states, is considered to be the endogenous production of inhibitors such as the family of Tissue Inhibitor of Metalloproteases (TIMPS), and alpha-2 macroglobulin. An imbalance between the local levels of the proteolytic enzymes and natural inhibitors will allow destruction of connective tissue components to occur.

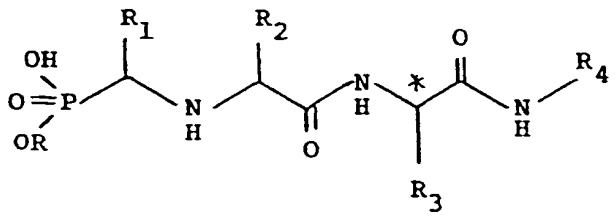
The compounds described in the present invention, being synthetic and low molecular weight inhibitors of this family of enzymes, offer a therapeutically useful way in which a more normal or non-pathological balance between inhibition and enzymic activity can be restored: they thus act to complement and supplement the endogenous enzyme inhibitors. Indeed, because these enzymes usually act only within restricted pericellular environments, before being inactivated by inhibitors circulating in the blood and present in most inflammatory exudates, the low molecular weight inhibitors disclosed here may be more

effective than endogenous proteinaceous inhibitors that are excluded by their size from the localized regions of connective tissue destruction.

European Patent Application 88310492.9 (Beecham Group) discloses a class of phosphorus derivatives having activity as inhibitors of collagenase and utility in the treatment of rheumatoid arthritis and related diseases in which collagenolytic activity is a contributing factor.

Novel structurally related compounds have now been discovered, which are collagenase inhibitors and thus of potential utility in the treatment of diseases in which collagenolytic activity and tissue remodelling is implicated.

According to the present invention there is provided a compound of general formula (I), or a salt, solvate or hydrate thereof:



(I)

in which,

R is hydrogen, C_{1-6} alkyl or optionally substituted benzyl;

R_1 is hydrogen or C_{1-6} alkyl;

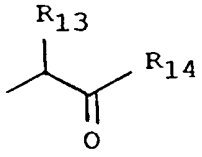
R_2 is C_{3-6} alkyl;

R_3 is $-(CH_2)_nNR_5R_6$, $-(CH_2)_nNHCOR_7$, $-(CH_2)_nCONH(CH_2)_qNR_5R_6$, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ or $-(CH_2)_n-R_{10}$ where n is an integer from 1 to 6 and each of R_5 and R_6 is independently

hydrogen or alkyl, or R_5 and R_6 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an optional oxygen or sulphur atom or an optionally substituted second nitrogen atom in the ring, R_7 is alkyl or $-(CH_2)_nNR_5R_6$, R_8 is hydrogen or alkyl, R_9 is hydrogen or alkyl or R_9 and R_5 together with the nitrogen atoms to which they are bonded form an optionally substituted 5-, 6- or 7-membered ring, and R_{10} is an optionally substituted piperidyl ring;

m is 1 or 2, and q is 2 to 4; and

R_4 is hydrogen, alkyl, and $-CH_2-(CH_2)_nOR_{11}$ or $-CH_2-(CH_2)_nOCOR_{12}$ or



where n is an integer from 1 to 6; R_{11}, R_{12} and R_{13} are hydrogen or C_{1-6}alkyl; and R_{14} is hydroxy or $-O-C_{1-6}$alkyl or $-NR_5R_6$ (where R_5 and R_6 may be linked to form a heterocyclic ring).

Unless otherwise specified, each alkyl group is preferably a C_{1-8} group, more preferably C_{1-6}, and may be a straight chain or branched.

R is preferably hydrogen, methyl or ethyl, especially hydrogen.

Values for R_1 include hydrogen, methyl, ethyl, isopropyl and n-butyl. As an alkyl group, R_1 is preferably methyl or ethyl.

R_2 is preferably a C_4 alkyl group, such as n-butyl, iso-butyl or sec-butyl, especially iso-butyl.

Values for R_3 include $-(CH_2)_nNR_5R_6$ where R_5 and R_6 are hydrogen or methyl, $-(CH_2)_nNHCOR_7$ where R_7 is $-(CH_2)_mNR_5R_6$ in which m is 1 and R_5 and R_6 are hydrogen, $-(CH_2)_nCONH(CH_2)_qNR_5R_6$ where q is 2 and R_5 and R_6 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ where R_5, R_6, R_8 and R_9 are all hydrogen, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ where R_5 and R_9 together with the nitrogen atoms to which they are bonded form an optionally substituted 2-imidazolinyl group, $-(CH_2)_nR_{10}$ where R_{10} is optionally substituted piperidyl, and n is an integer from 1 to 4.

Most preferably R_3 is $-(CH_2)_nNR_5R_6$ where n is 3 or 4 and R_5 and R_6 are both hydrogen or methyl, $-(CH_2)_4NHCOR_7$ where R_7 is $-CH_2NH_2$, $-CH_2CONH(CH_2)_2NR_5R_6$ where R_5 and R_6 are joined together to form a pyrrolidine ring, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ where n is 3 or 4 and R_5, R_6, R_8 and R_9 are all hydrogen, $-(CH_2)_4NR_8C(=NR_9)NR_5R_6$ where R_5 and R_9 together with the nitrogen atoms to which they are bonded form an optionally substituted 2-imidazolinyl group and R_6 and R_8 are both hydrogen, $-(CH_2)_nNHC(=NH)NH_2$ where n is 3 or 4, and $-CH_2R_{10}$ where R_{10} is 4-piperidyl.

Preferred values for R_4 are methyl, ethyl, $-(CH_2)_2OCH_3$, $-CH(CH_3)CO_2CH_3$ and $-(CH_2)_2OH$, especially methyl and $-(CH_2)_2OH$.

The compounds of formula (I) may form salts with bases e.g. sodium hydroxide. The compounds of formula (I) have a basic nitrogen atom and may form acid addition salts e.g. with hydrochloric acid. Such compounds form part of the present invention.

Where compounds of formula (I), or salts thereof, form solvates or hydrates, these also form an aspect of the invention.

The compounds of formula (I) have at least two, and may have three or more asymmetric centres and therefore exist in more than one stereoisomeric form. The invention extends to all such forms and to mixtures thereof, including racemates, and diastereoisomeric mixtures.

Preferred isomers are those having the S-configuration at the chiral centre bearing R_2 and the S-configuration at the chiral centre bearing R_3, marked with an asterisk in formula (I).

The compounds of formula (I) or their salts, solvates or hydrates are preferably in pharmaceutically acceptable form. By pharmaceutically acceptable form is meant, inter alia, of a pharmaceutically acceptable level of purity excluding normal pharmaceutical additives such as diluents and carriers, and including no material considered toxic at normal dosage levels.

The compounds of formula (I) or their salts, solvates or hydrates are preferably in substantially pure form. A substantially pure form will generally contain at least 50% by weight, preferably 75%, more preferably 90% and still more preferably 95% or 99% or more of the compound of formula I or its salt or solvate.

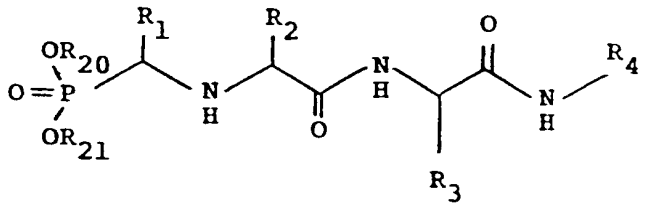
Compounds of formula (I) or their salts, solvates or hydrates may be isolated as crystalline solids or in the form of foams or gums.

A preferred pharmaceutically acceptable form is the crystalline form.

The present invention provides the compounds of formula (I) or pharmaceutically acceptable salts, solvates or hydrates thereof for use as active therapeutic agents, particularly as agents for treatment of conditions in which degradation of connective tissue and other proteinaceous components of the body occurs, such as musculo-skeletal disorders resulting from collagenolytic activity, particularly rheumatism and/or arthritic conditions, and tissue remodelling.

Compounds of formula (I) also have potential utility in the treatment of cancer; for preventing myelin degradation in the central and peripheral nervous system; and in other conditions in which members of the collagenase family of neutral metalloproteases have pathological or other roles.

The present invention also provides a process for the preparation of a compound of formula (I) which comprises converting a group R_{20} to hydrogen by cleaving a group R_{20} from a compound of formula (II):



(II)

wherein R_{20} is alkyl, optionally substituted phenyl or optionally substituted benzyl and R_{21} is hydrogen, alkyl, optionally substituted phenyl or optionally substituted benzyl and R_1, R_2, R_3 and R_4 are as defined in formula (I), and where necessary, converting R_{21} to hydrogen, and optionally thereafter converting the compound of formula (I) to a further compound of formula (I).

Cleavage of R_{20}, and where necessary R_{21}, may be carried out in aqueous acid or alkali or using a trimethylsilyl halide, preferably bromotrimethylsilane, in an inert solvent, for example dichloromethane or acetonitrile. Benzyl esters may alternatively be removed by hydrogenolysis or other standard debenzylation procedures. Phenyl residues may be removed by hydrogenation over platinum oxide.

When both R_{20} and R_{21} are alkyl, cleavage of R_{20} only, to give to a compound of formula (II) in which R_{20} is hydrogen and R_{21} alkyl, which is a compound of formula (I) in which R is alkyl, may be carried out by treatment with excess alkali under mild conditions, for example with aqueous sodium hydroxide in an alcoholic solvent at room temperature.

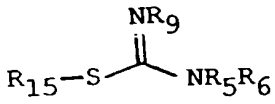
Similarly, where R_{20} is optionally substituted benzyl and R_{21} is alkyl, the benzyl group only may be cleaved by hydrogenation to give a compound of formula (II) in which R_{20} is hydrogen and R_{21} is alkyl.

Cleavage of an R_{21} alkyl group may thereafter be carried out as described above to give a compound of formula (I) in which R is hydrogen.

When R in a compound of formula (I) is hydrogen and R_{21} in a compound of formula (II) is not hydrogen, then cleavage of both R_{21} and R_{20} is conveniently effected in a single reaction. Preferably R_{20} and R_{21} are both alkyl, such as methyl or ethyl, or benzyl.

It will be appreciated that compounds of formula (II) in which R_{21} is hydrogen are themselves compounds of the invention of formula (I).

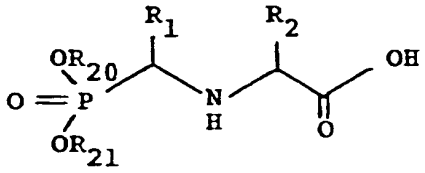
A compound of formula (I) in which R_3 is $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ in which R_5, R_6, R_8 and R_9 are as defined in formula (I) may be prepared by reacting a compound of formula (I) in which R_3 is $-(CH_2)_nNR_5R_6$ in which R_5 and R_6 are either both hydrogen or one is hydrogen and the other is alkyl with a compound of formula (IIA)



(IIA)

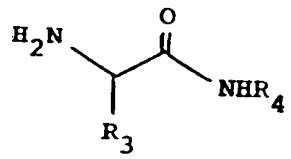
or a salt thereof in which R_5, R_6 and R_9 are as defined in formula (I) and R_{15} is C_{1-6}alkyl. The reaction may be carried out in the presence of a base such as sodium bicarbonate in a suitable solvent such as water.

Compounds of formula (II) may be prepared by treating a compound of formula (III):



(III)

in which R_1, R_2, R_{20} and R_{21} are as defined in formula (II) (except that R_{21} is not H), with a compound of formula (IV):



(IV)

in which R_3 and R_4 are as defined in formula (I), and any reactive amine group in R_3 is in protected form.

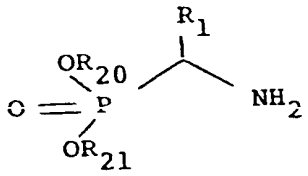
The reaction is preferably carried out in the presence of a coupling agent, such as dicyclohexylcarbodiimide or 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride in the presence of 1-hydroxybenzotriazole, or using 1,1'-carbonyldiimidazole, in an inert solvent such as dichloromethane or acetonitrile.

Compounds of the formula (II) in which R_3 is $-(CH_2)_n-R_{10}$ where n and R_{10} are as defined in formula (I) can be prepared from the compound of formula (II) in which R_3 is $-(CH_2)_n-Z$ where n is as defined in formula (I) and Z is an optionally substituted pyridyl ring, by hydrogenation in the presence of a noble metal catalyst.

It will be appreciated that when R_3 is in protected form, the protecting group may be chosen to undergo concomitant cleavage with R_{20} and/or R_{21}.

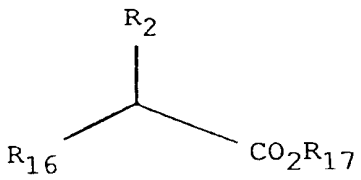
Selective cleavage of the group R_{21} may then be carried out using the procedures described above for the preparation of compounds of formula (I) to give compounds of formula (II) in which R_{21} is hydrogen.

The intermediate compounds of formula (III) may be prepared by treating a compound of formula (V) or a salt thereof:

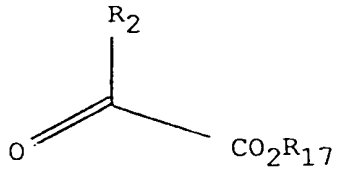


(V)

in which R_1, R_{20} and R_{21} are as defined in formula (III), with a compound of formula (VIA) or (VIB) or a salt thereof:



(VIA)



(VIB)

in which R_2 is as defined in formula (I), R_{16} is a leaving group such as halogen, methanesulphonyloxy or trifluoromethanesulphonyloxy and R_{17} is hydrogen or a carboxyl protecting group, and thereafter removing an R_{17} carboxyl protecting group. The preferred method is the reaction of (V) with (VIA).

When a compound of formula (VIB) is used, the reductive amination may be carried out by hydrogenation over a noble metal catalyst such as palladium on carbon or by reaction with sodium cyanoborohydride at pH 6 to 7. Lower alkyl alcohol solvents such as methanol and ethanol are suitable for both reactions. These reactions may be carried out in the presence of molecular sieves.

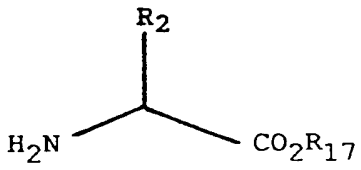
A hydrogenation reaction is preferred but this process precludes the use of compounds of formulae (V) and (VIB) in which any of R_{20}, R_{21} or R_{17} is benzyl. Preferably a carboxyl protecting group is a methyl or ethyl ester. Ester protecting groups may be removed under standard basic hydrolysis conditions using dilute base such as 1 Normal aqueous sodium hydroxide in methanol, or aqueous potassium hydroxide in 1,4-dioxane.

When the compound of formula (V) is in the form of the free base, the compound of formula (VIB) is suitably an α-keto ester (R_{17} = alkyl).

When the compound of formula (V) is a salt, such as the hydrochloride salt, the compound of formula (VIB) is suitably a salt of an α-keto acid (R_{17} = H), for example the sodium salt.

The preparation of compounds of formula (III) using a compound of formula (VIA) may be carried out under standard alkylation conditions. A halogen leaving group is preferably bromine and an oxygen-based leaving group is preferably trifluoromethanesulphonyloxy.

Compounds of formula (III) may alternatively be prepared by condensing a compound of formula (VII) or a salt thereof:

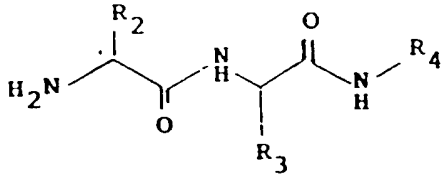


(VII)

in which R_2 is as defined in formula (I) and R_{17} is a carboxyl protecting group with an aldehyde, R_1-CHO in which R_1 is as defined in formula (I) and treating the condensation product with an appropriate dialkyl or trialkyl phosphite, for example dimethyl phosphite, and thereafter removing the carboxyl protecting group. The carboxyl group is conveniently protected as an alkyl or benzyl ester which may be removed using standard hydrolysis or hydrogenation conditions.

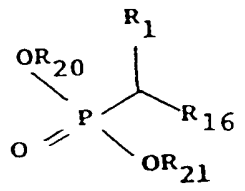
As described above in connection with reductive amination of compounds of formula (VIB), where a benzyl protecting group R_{17} is removed by hydrogenation then R_{20} and R_{21} are restricted to alkyl.

Alternatively, compounds of formula (II) in which R_{20} and R_{21} are alkyl or optionally substituted benzyl may be prepared by the reaction of a compound of formula (VIII):



(VIII)

in which R_2, R_3 and R_4 are as defined in formula (I), with a compound of formula (IX):



(IX)

in which R_1 is as defined in formula (I), R_{20} and R_{21} are alkyl, optionally substituted phenyl, or optionally substituted benzyl and R_{16} is a leaving group as defined for formula (VIA), in the presence of a base such as triethylamine or Proton Sponge (1,8-bis(dimethylamino)-naphthalene), or using anhydrous potassium carbonate in an alcoholic solvent.

Where R_{16} is an oxygen-based leaving group, for example trifluoromethanesulphonyloxy, which is preferred, displacement of the leaving group is conveniently carried out in the presence of Proton Sponge in an inert solvent such as acetonitrile or dichloromethane, over a period of several days in the absence of light.

A further alternative preparation of compounds of formula (III) may be carried out by reacting a compound of formula (IX) as hereinbefore defined with a compound of formula (VII) in which R_{17} is a carboxyl protecting group, using conditions as described for the reaction of compounds of formula (VIII) with compounds of formula (IX), and thereafter removing the protecting group R_{17}.

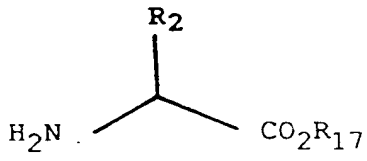
Suitable carboxyl protecting groups include alkyl, benzyl, trialkylsilyl, and trialkylsilylethyl groups. A trialkylsilyl protecting group, for example trimethylsilyl, is especially useful in that it may be readily incorporated, in situ, for example by addition of hexamethyldisilazane to the reactants in acetonitrile in the presence of triethylamine, and selectively removed in aqueous methanol, without imposing any limitations on the value of R_{20} and R_{21}. Other silylating agents include trimethylsilyl chloride and N,N-diethyltrimethylsilylamine.

An R_{17} alkyl carboxyl protecting group may be removed by base hydrolysis, for example using sodium hydroxide in aqueous methanol or potassium hydroxide in aqueous 1,4-dioxane.

It will be appreciated that where the carboxyl protecting group R_{17} is alkyl, R_{20} and R_{21} may be alkyl, phenyl or benzyl derivatives, but where R_{17} is a benzyl group, R_{20} and R_{21} are limited to alkyl and phenyl.

When compounds of formula (III) are prepared by this route, it is preferred that R_{20} and R_{21} are benzyl and R_{16} is trifluoromethanesulphonyloxy in the compound of formula (IX) and R_{17} is trimethylsilyl or methyl in the compound of formula (VII).

Compounds of formula (VIII) may be prepared by treating a compound of formula (VII):



(VII)

in which R_2 is as defined in formula (I), R_{17} is hydrogen and wherein the amino group is optionally protected, with a compound of formula (IV) as hereinbefore defined, in the presence of a coupling agent as hereinbefore described for the preparation of compounds of formula (II) from compounds of formulae (III) and (IV).

Compounds of formula (IX) may be prepared from hydroxyalkylphosphonate derivatives by conversion of the hydroxyl group to the leaving group R_{16} by conventional methods. For example, where R_{16} is trifluoromethanesulphonyloxy, trifluoromethanesulphonic anhydride may be added to a solution of the hydroxyalkylphosphonate in an inert solvent such as dichloromethane, the reaction being carried out at reduced temperature under an inert atmosphere, according to the general method of E. Vedejs et al., Journal of Organic Chemistry 50, 2165, (1985).

Hydroxyalkylphosphonate compounds may in turn be prepared by reaction of the corresponding phosphite, for example dibenzylphosphite, with an aldehyde R_1-CHO in which R_1 is as defined in formula (I) according to the general method of F. Texier-Boullet and A. Foucaud, Synthesis, 916 (1982). Benzyl and alkyl phosphites are either commercially available compounds or can be prepared from commercially available starting materials by standard methods.

Intermediate compounds of formula (V) are either known compounds or may be prepared from known aminoalkyl phosphonic acid derivatives using standard procedures to introduce R_{20} and R_{21} as required.

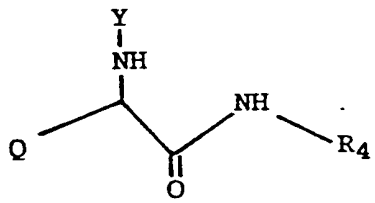
Protection of the amine function during these reactions may be necessary.

Introduction of an R_{20} or R_{21} methyl group may be effected by reaction with diazomethane in a suitable inert solvent.

Compounds of formula (V) of fixed configuration may be prepared by the general method of R. Jacquier et al., Phosphorus and Sulfur 36, 73, (1988).

Compounds of formula (IV) may be prepared from amino acid derivatives, many of which are commercially available, by conventional alkylation or coupling reactions.

Thus a compound of formula (IV) may be prepared from a compound of formula (X):



(X)

in which Q is $-(CH_2)_n-Z$, $-(CH_2)_nNH_2$, $-(CH_2)_nNR_8C(=NH)NH_2$, $-(CH_2)_nNR_8C(=NH)NO_2$ or $-(CH_2)_nCO_2H$, n, R_4 and R_8 are as defined in formula (I), Z is optionally substituted pyridyl and Y is a nitrogen protection group, by conversion of Q to R_3 and removal of the nitrogen protection group, Y. It will be appreciated that the conversion of Q to R_3 may be most readily effected at a later stage, for example conversion of $(CH_2)_nNHC(=NH)NHNO_2$ to $(CH_2)_nNHC(=NH)NH_2$ by hydrogenation can be concomitant with hydrogenolysis of R_{20} and R_{21} benzyl groups.

A compound of formula (IV) in which R_3 is $-(CH_2)_nNR_5R_6$ may be prepared by alkylation of a compound of formula (X) in which Q is $-(CH_2)_nNH_2$ using standard alkylation procedures.

A compound of formula (IV) in which R_3 is $-(CH_2)_nNHCOR_7$ may be prepared by reaction of a compound of formula (X) in which Q is $-(CH_2)_nNH_2$ with a carboxylic acid R_7CO_2H, in the presence of a coupling agent.

A compound of formula (IV) in which R_3 is $-(CH_2)_nCONH(CH_2)_qNR_5R_6$ may be prepared by reaction of a compound of formula (X) in which Q is $-(CH_2)_nCO_2H$ with an amine derivative, $NH_2(CH_2)_qNR_5R_6$ in the presence of a coupling agent, and thereafter if R_5 or R_6 is hydrogen optionally protecting the basic nitrogen atom.

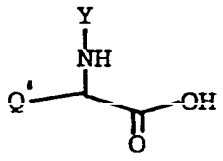
A compound of formula (IV) in which R_3 is $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ may be prepared from a compound of formula (X) in which Q is $-(CH_2)_nNR_8C(=NH)NH_2$ or $-(CH_2)_nNH_2$ by N-alkylation and optionally thereafter protecting the basic nitrogen atoms.

A compound of the formula (IV) in which R_3 is $-(CH_2)_n-R_{10}$ where R_{10} is a piperidyl group may be prepared by hydrogenation of a compound of formula (X) in which R_3 is $-(CH_2)_n-Z$ and optionally thereafter protecting the piperidyl nitrogen atom.

Suitable nitrogen protection groups for Y and for any primary amino function in R_3 include t-butoxycarbonyl (BOC) and benzyloxycarbonyl groups. When R_3 is $-(CH_2)_n-R_{10}$ where R_{10} is 4-piperidyl, a suitable nitrogen protecting group includes the benzyloxycarbonyl group.

Nitrogen protection groups may be removed by standard methods. A t-butoxycarbonyl group may be removed by treatment with trifluoroacetic acid at reduced temperature. Benzyloxycarbonyl groups may be removed by catalytic hydrogenation.

A compound of formula (X) may be prepared from a compound of formula (XI):



(XI)

wherein Q' is Q in protected form or Q' is a precursor to Q and Y and Q are as defined for formula (X).

The reaction may be carried out by reaction with an amine, NH_2R_4, using standard procedures for forming an amide from a carboxylic acid and an amine, for example using a coupling agent such as 1,1'-carbonyldiimidazole, 1,3-dicyclohexylcarbodiimide or 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, or in the presence of ethyl chloroformate.

Compounds of formula (XI) are known compounds or may be prepared from known starting materials by standard methods.

For example the compound of formula (XI) in which Q' is $(CH_2)_4NHC(O)OCH_2Ph$ and Y is t-butoxycarbonyl is derived from lysine and is commercially available.

The compound of formula (XI) in which Q' is $CH_2CO_2CH_2Ph$ and Y is t-butoxycarbonyl is derived from aspartic acid and is commercially available.

Compounds of formula (IIA) and (IIB) are commercially available or may be prepared from known starting materials using standard procedures.

The compound of formula (XI) in which Q' is a group $-(CH_2)_n-Z$ where Z is 4-pyridyl and Y is t-butoxycarbonyl is prepared according to the method of R.L. Bixler and C. Niemann, J. Org. Chem., 23, 575 (1958).

The compounds of formula (VII) are either known amino acid derivatives or can be made from these derivatives by known methods. Compounds of formula (VIA) and (VIB) are either known compounds or may be prepared from known compounds by known methods.

The intermediates of formula (II) disclosed herein are novel compounds and form an aspect of the present invention as do the described processes for their preparation.

Where obtainable, pharmaceutically acceptable salts of the compounds of formula (I) may be formed conventionally by reaction with the appropriate acid or base. Solvates may be formed by crystallization from the appropriate solvent.

As mentioned previously, the compounds of formula (I) exist in more than one diastereoisomeric form. Where the processes of the invention produce mixtures thereof, the individual isomers may be separated one from another by chromatography e.g. HPLC.

Alternatively, separate diastereoisomeric compounds of formula (I) can be obtained by using stereoisomerically pure starting materials or by separating desired isomers of intermediates at any stage in the overall synthetic process, and converting these intermediates to compounds of formula (I).

It will be appreciated that where a single diastereoisomer of a compound of formula (I) is prepared by more than one process variant as hereinbefore described, each of which allows a different chiral centre to be defined, it may be possible to deduce the configuration at a chiral centre which is not pre-determined using a particular process variant.

Furthermore, it will be appreciated that although the absolute configuration at a particular chiral centre may not be known, it is possible to characterise a given diastereoisomer relative to its epimer by reference to the direction in which the plane of polarised light is rotated.

The present invention further provides a pharmaceutical composition, which comprises a compound of formula (I), or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier.

A composition of this invention is useful in the treatment of musculo-skeletal disorders, particularly arthritic diseases and for modulation of tissue remodelling.

A composition of the invention also has potential utility in the treatment of cancer; for preventing myelin degradation in the central and peripheral nervous system;

and in other conditions in which members of the collagenase family of neutral metalloproteases have pathological or other roles.

A composition of the invention, which may be prepared by admixture, may contain a diluent, binder, filler, disintegrant, flavouring agent, colouring agent, lubricant or preservative in conventional manner. These conventional excipients may be employed in conventional manner, for example as in the preparation of compositions of related peptide enzyme inhibitors, such as the ACE inhibitor enalapril.

A composition of the invention may be adapted for oral, topical, rectal or parenteral administration but oral administration is preferred. Parenteral compositions may be administered intravenously, intramuscularly, intra-articularly, intradermally, subcutaneously or into the cerebro-spinal fluid.

Preferably, a pharmaceutical composition of the invention is in unit dosage form and in a form adapted for use in the medical or veterinarial fields. For example, such preparations may be in a pack form accompanied by written or printed instructions for use as an agent in the treatment or prophylaxis of any of the disorders mentioned above.

The suitable dosage range for the compounds of the invention may vary from compound to compound and may depend on the condition to be treated. It will also depend, inter alia, upon the relation of potency to absorbability and the mode of administration chosen.

The compound or composition of the invention may be formulated for administration by any route, the preferred route depending upon the disorder for which treatment is required, and is preferably in unit dosage form or in a form that a human patient may administer to himself in a single dosage.

Compositions may, for example, be in the form of tablets, capsules, sachets, vials, powders, granules, lozenges, reconstitutable powders, or liquid preparations, for example solutions or suspensions, or suppositories.

The compositions, for example those suitable for oral administration, may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate; disintegrants, for example starch, polyvinylpyrrolidone, sodium starch glycollate or microcrystalline cellulose; or pharmaceutically acceptable wetting agents such as sodium lauryl sulphate.

Solid compositions may be obtained by conventional methods of blending, filling, tableting or the like. Repeated blending operations may be used to distribute the active agent throughout those compositions employing large quantities of fillers. When the composition is in the form of a tablet, powder, or lozenge, any carrier suitable for formulating solid pharmaceutical compositions may be used, examples being magnesium stearate, starch, glucose, lactose, sucrose, rice flour and chalk. Tablets may be coated according to methods well known in normal pharmaceutical practice, in particular with an enteric

coating. The composition may also be in the form of an ingestible capsule, for example of gelatin containing the compound, if desired with a carrier or other excipients. For example, in a hard gelatin capsule containing the required amount of a compound of the invention in the form of a powder or granulate in intimate mixture with a lubricant, such as magnesium stearate, a filler, such as microcrystalline cellulose, and a disintegrant, such as sodium starch glycollate.

Compositions for oral administration as liquids may be in the form of, for example, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid compositions may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; aqueous or non-aqueous vehicles, which include edible oils, for example almond oil, fractionated coconut oil, oily esters, for example esters of glycerine, or propylene glycol, or ethyl alcohol, glycerine, water or normal saline; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

The compounds of this invention may also be administered by a non-oral route. In accordance with routine pharmaceutical procedure, the compositions may be formulated, for example for rectal administration as a suppository or for parenteral administration in an injectable form. For injection, for example by intra-articular injection or by injection into the cerebro-spinal fluid or via other routes which will gain

access to sites of demyelination, such as by intramuscular, intradermal or subcutaneous injection, as freely soluble solutions or as poorly dispersed depot stores, the compounds of the invention may be presented in an aqueous or non-aqueous solution, suspension or emulsion in a pharmaceutically acceptable liquid, e.g. sterile pyrogen-free water or a parenterally acceptable oil or a mixture of liquids, which may contain bacteriostatic agents, anti-oxidants or other preservatives, buffers or solutes to render the solution isotonic with the blood, thickening agents, suspending agents or other pharmaceutically acceptable additives. Such forms will be presented in sterile unit dose form such as ampoules or disposable injection devices or in multi-dose forms such as a bottle from which the appropriate dose may be withdrawn or a solid form or concentrate which can be used to prepare an injectable formulation.

For topical and percutaneous administration, the preparations may also be presented as an ointment, cream, lotion, gel, spray, aerosol, wash, skin paint or patch.

A unit dose for treating diseases in which enzymes of the collagenase family are involved will generally contain from 10 to 1000 mg and preferably will contain from 10 to 500 mg, in particular 10, 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 mg. The composition may be administered one or more times a day, for example 2, 3 or 4 times daily, so that the total daily dose for a 70 kg adult will normally be in the range 10 to 3000 mg. Such a dosage corresponds to approximately 0.15 to 50 mg/kg per day. Alternatively, in particular for injection, the unit dose will contain from 2 to 200 mg of a compound of the invention and be administered in multiples, if desired, to give the desired daily dose.

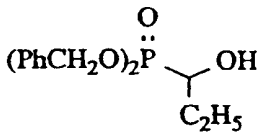
The present invention additionally provides a method of treating conditions in which degradation of connective tissue and other proteinaceous components of the body occurs, such as rheumatism and/or arthritic conditions in mammals, such as humans, which comprises administering to the mammal in need of such treatment an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt thereof.

The present invention also provides the use of a compound of formula (I) or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for use in the treatment of conditions in which degradation of connective tissue and other proteinaceous components of the body occurs such as rheumatism and/or arthritic conditions.

The following Descriptions and Examples illustrate the preparation of compounds of the invention. All temperatures are expressed in °C.

Description 1

Dibenzyl (1-hydroxypropyl)phosphonate (D1)



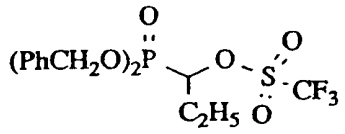
The general method of F. Texier-Boullet and A. Foucaud [Synthesis, 1982, 916] was employed. A mixture of dibenzyl phosphite (31.13 ml, 0.14 mole) and propionaldehyde (10.21 ml, 1 equiv.) was stirred at room temperature and basic alumina (70g) added in one portion. After standing overnight at room temperature chloroform was added and the alumina collected and washed with chloroform. The filtrate was evaporated to dryness and the resulting clear oil chromatographed on silica gel (600g) with gradient elution (ether - 5% methanol/ether). The title compound was obtained as a clear oil which solidified on standing (27.82g, 64%). A sample was recrystallized from ether/pentane to give a white crystalline solid, m.p. 81-82°C.

Found: C,64.09; H,6.71. $C_{17}H_{21}O_4P_1$ requires C,63.74; H,6.61%.

δ ($CDCl_3$): 1.04(3H,t,J=7Hz), 1.6-1.95(2H,m), 2.27(1H, br s), 3.8(1H, 2 overlapping triplets, J=5 and 10Hz), 4.97-5.18(4H,m), 7.34(10H,s).

Description 2

Dibenzyl ((1-trifluoromethanesulphonyloxy)propyl)phosphonate (D2)

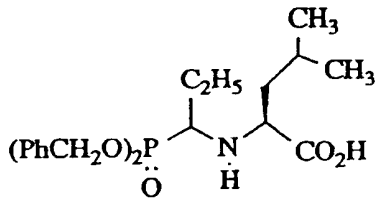


The title compound was prepared by the general method of E. Vedejs et al. [J. Org. Chem. 1985, 50(12), 2165]. A solution of dibenzyl (1-hydroxypropyl)phosphonate (D1) (24.97g, 0.078 mole) in methylene chloride (180 ml) was cooled to -50°C under N_2. 2,6-Lutidine (11.12 ml, 0.095 mole) was added followed by trifluoromethanesulphonic anhydride (15.1 ml, 0.0898 mole) keeping the temperature at -50°C. The mixture was allowed to warm slowly to 0°C and then taken into cold ether. The solution was subjected to a rapid aqueous work-up by washing the organic layer with ice-cold water, dilute hydrochloric acid (x2) and finally brine. The organic layer was dried (anhydrous $MgSO_4$) and evaporated to dryness to give the title compound as a pinkish orange oil (33.77g, 96%) which was used without further purification.

δ ($CDCl_3$): 1.08(3H,t,J=7Hz), 1.88(2H,m), 4.94(1H, 2 overlapping triplets,J=5.5 and 7Hz), 4.88-5.22(4H,m) and 7.35(10H,m).

Description 3

N-(1-(R)-Dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) and N-(1-(S)-Dibenzyloxyphosphinylpropyl)-(S)-leucine (D3B)



Method A

Following the general method of US 4808741 for the preparation of leucine trimethylsilyl ester a mixture of (S)-leucine (1.15g, 0.0088 mole), hexamethyldisilazane (1.75 ml), and triethylamine (1.38 ml) in acetonitrile (13.5 ml) was heated at reflux for a total of 4h.

Dibenzyl ((1-trifluoromethanesulphonyloxy)propyl)-phosphonate (D2) (4.5g, 0.01 mole) was then added and the mixture maintained at 40-42°C for 48h. The reaction can also be carried out at ambient temperature. After cooling the mixture was filtered, washed with methanol and the filtrate evaporated to dryness. The residue was taken up in chloroform and washed with dilute HCl (x2) and finally water. The chloroform layer was dried (anhydrous Na_2SO_4), filtered and evaporated to dryness to give an orange gummy solid (3.67g). The crude product was triturated with the minimum volume of ether/pentane to give a white crystalline solid which after collection, washing with a little cold ether/pentane and drying gave the title compound, R,S isomer (D3A) (0.47g, 11%), m.p. 112-115°C.

Observed Desorption CI (NH_3) $(M+H)^+$ 434. $C_{23}H_{32}NO_5P$ requires M 433.

$[\alpha]_D^{20}$ = -23.09° (c=0.97 MeOH).

Found: C,63.73; H,7.42; N,3.23. $C_{23}H_{32}NO_5P$ requires C,63.73; H,7.44; N,3.23%.

δ ($CDCl_3$): 0.89 (6H,t), 1.03 (3H,t), 1.25-2.0 (5H,m), 2.74 (1H,m), 3.28 (2H, br s), 3.73 (1H, br t), 4.9-5.15 (4H, m), 7.35 (10H, s).

The other isomer, N-(1-(S)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3B), can be obtained by preparative HPLC using a Hamilton PRP-1 column, 300 x 7.0mm, 264R with a 40:60 acetonitrile:water eluent mixture and a flow rate of 4.0 ml/min. Under these conditions the R,S isomer (D3A) elutes first with a retention time of 34.6 min and the S,S isomer (D3B) is well separated at 42.7 min.

For the isomer (D3B):

Observed FAB $(M+H)^+$ 434. $C_{23}H_{32}NO_5P$ requires M 433.

δ ($CDCl_3$): 0.88 (6H,dd), 0.98 (3H,t), 1.4 (1H,m), 1.52-1.9 (4H,m), 2.72 (1H,m), 3.38 (1H,m), 4.9-5.15 (4H,m), 7.32 (10H,s).

The S,S isomer (D3B) on coupling with (S)-amino acid derivatives leads to the S,S,S, series.

Method B

A mixture of (S)-leucine methyl ester hydrochloride (0.543g; 0.003 mole), dibenzyl (1-trifluoromethane-sulphonyloxy)propyl)-phosphonate (D2) (1.35g; 0.003 mole) and anhydrous potassium carbonate (1.0g) in methanol (2 ml) was heated at 50°C, with stirring, for 4 hours and then left at room temperature overnight.

The reaction mixture was evaporated to dryness in vacuo, and dissolved in chloroform (5 ml) and filtered. The filtrate, and washings, were combined and chromatographed on silica gel 60 (50g) using ethyl acetate-pentane (1:1) as the eluent, to afford a mixture of N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine methyl ester and N-(1-(S)-dibenzyloxyphosphinylpropyl)-(S)-leucine methyl ester as an oil (0.55g). The esters can be separated into the individual diastereoisomers by column chromatography on silica gel with initially 50% diethyl ether/pentane as eluent, rising to 100% diethyl ether.

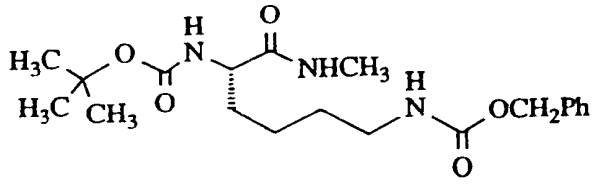
The above mixture of esters (1.1g, 0.0025 mole) in methanol (4.0 ml) was treated with a solution of sodium hydroxide (0.11g; 0.00275 mole) in water (1.5 ml), and the solution was stirred at room temperature overnight. It was evaporated to one third volume, in vacuo, taken in water and extracted with ether. The aqueous fraction was acidified with citric acid to pH 3-4 and then extracted (5x) with chloroform. The chloroform fraction was dried (Na_2SO_4) and evaporated to dryness in vacuo to give a mixture of the title compounds (D3A) and (D3B) as an oil that slowly solidified.

Trituration of the product with ether gave N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (0.34g) as a white crystalline solid, identical to the product obtained by Method A.

Alternatively, the single isomer esters can be hydrolysed separately. For example N-(1-(S)-dibenzyloxyphosphinylpropyl)-(S)-leucine methyl ester on hydrolysis by the above method gave N-(1-(S)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3B), m.p. 71-73°C.

Description 4

N^{α}-tert-Butoxycarbonyl-N^{ε}-benzyloxycarbonyl-(S)- lysine methylamide (D4)

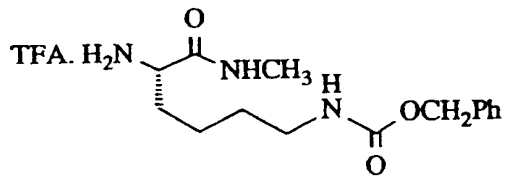


A stirred solution of N^{α}-tert-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-(S)-lysine (1.5g, 3.95 mmol) in anhydrous dichloromethane (30 ml) maintained at 0°C was sequentially treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.91g, 4.7 mmol) and 1-hydroxybenzotriazole (0.64g, 4.7 mmol). After 0.5 h, the mixture was warmed up to room temperature, methylamine bubbled through, and the resulting solution left stirring for 18 h. The solution was then filtered, washed with sat. aq. $NaHCO_3$, dried over anhydrous magnesium sulphate and concentrated under reduced pressure to afford a solid. Purification by flash chromatography (5% methanol in chloroform) gave the title compound as a white solid (1.18g).

δ ($CDCl_3$): 1.3-1.85 (6H,m), 1.42 (9H,s), 2.28 (3H,d), 3.19 (2H,q), 4.05 (1H,m), 4.88 (1H,m), 5.08 (2H,s), 5.15 (1H,m), 6.2 (1H,m), 7.35 (5H,m).

Description 5

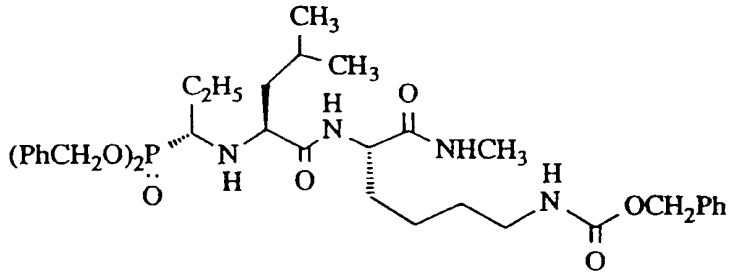
N^{ε}-Benzyloxycarbonyl-(S)-lysine methylamide, trifluoroacetate salt (D5)



A cooled (0°C) solution of the amide (D4) (0.3g, 0.76 mmol) in dichloromethane (5 ml) was treated with trifluoroacetic acid (2 ml). After 0.5 h the solvent was evaporated under reduced pressure, the residue diluted with dichloromethane (15 ml) and washed with sat. aq. NaCl (10 ml). The organic fraction was dried over anhydrous magnesium sulphate and evaporated in vacuo to give crude title compound (D5) (0.29g). This was used as such without further purification.

Description 6

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methylamide (D6)

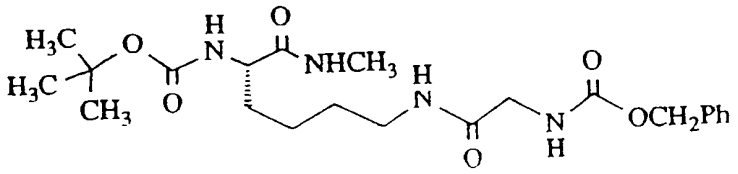


A solution of N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (0.33g, 0.76 mmol) in anhydrous dichloromethane (10 ml) was cooled to 0°C, and treated sequentially with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.176g, 0.92 mmol) and 1-hydroxy-benzotriazole (0.124g, 0.92 mmol). After stirring for 0.5 h the reaction mixture was treated with the salt (D5) (0.29g) followed by N,N-diisopropylethylamine (0.216g, 1.42 mmol). The mixture was stirred for 18 h at room temperature, then washed with sat. aq. $NaHCO_3$ (2x20 ml), and sat. aq. NaCl (2x20 ml). The aqueous washes were back-extracted with dichloromethane and the combined organic fractions dried over anhydrous magnesium sulphate, and evaporated in vacuo to yield an oil. On purification by flash chromatography [(MeOH:CH_2Cl_2)(1:20) v/v then (1:9) v/v], the title compound was isolated as a white solid (0.20g).

Observed FAB $(M+H)^+$ 678. $C_{38}H_{53}O_7N_4$ requires M 677.

Description 7

N^{α}-tert-Butoxycarbonyl-N^{ε}-(N-benzyloxycarbonyl-glycyl)-(S)-lysine methylamide (D7)

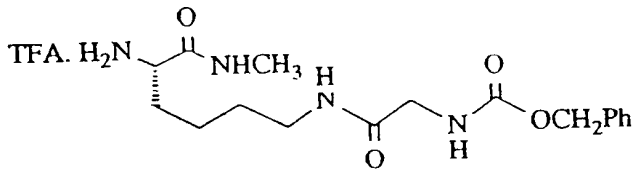


A stirred solution of N-benzyloxycarbonyl-glycine (1.8g, 5.82 mmol) in anhydrous dichloromethane (60 ml) was treated with 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (1.23g, 6.4 mmol) followed by 1-hydroxybenzotriazole (0.98g, 7.2 mmol). Stirring was continued for 0.5 h then treated with N^{α}-tert-butoxycarbonyl-(S)-lysine methylamide (1.51g, 5.82 mmol) diluted in dichloromethane (5 ml), and left stirring. After 18 h the mixture was washed with sat. aq. $NaHCO_3$ (2x30 ml), dried over anhydrous magnesium sulphate and evaporated in vacuo to yield a pale yellow oil. Purification by flash chromatography afforded the title compound as a clear viscous oil which solidified on standing.

δ ($CDCl_3$): 1.26-1.82 (6H,m), 1.42 (9H,s), 2.79 (3H,d), 3.26 (2H,m), 3.87 (2H,d), 4.05 (1H,m), 5.12 (2H,s), 5.28 (1H,m), 5.72 (1H,brs), 6.46 (1H,brs), 7.32-7.4 (5H,m).

Description 8

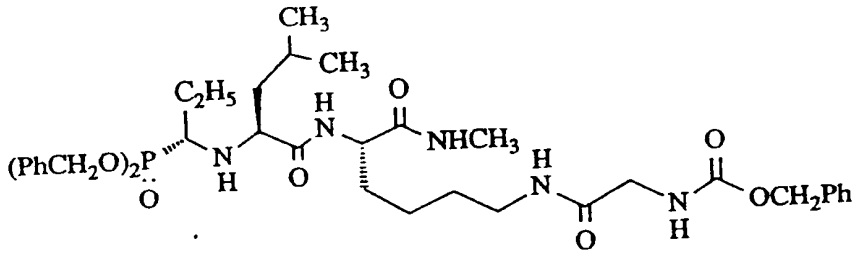
N^{ε}-(N-Benzyloxycarbonylglycyl)-(S)-lysine methylamide, trifluoroacetate salt (D8)



A cool (0°C) solution of the amide (D7) (0.1g, 0.22 mmol) in dichloromethane (5 ml) was treated with trifluoroacetic acid (2 ml). After stirring at 0°C for 1 h, the solution was allowed to warm up to room temperature. The solvent was evaporated under reduced pressure, to yield the crude product (D8) which was used without further purification.

Description 9

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-N^{ε}-(N-benzyloxycarbonylglycyl)-(S)-lysine methylamide, dibenzyl ester (D9)



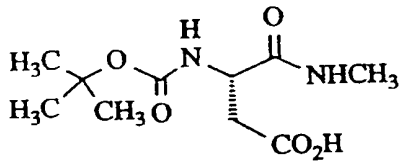
A solution of N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (0.43g, 1 mmol) in anhydrous dichloromethane (20 ml) was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.21g, 1.1 mmol) and 1-hydroxybenzotriazole (0.162g, 1.2 mmol). After stirring for 1 h, the salt (D8) (0.25g), diluted in dichloromethane (10 ml), was added followed by diisopropylethylamine (1 equiv.) stirring continued for a further 18 h. The mixture was then washed with sat. aq. $NaHCO_3$ (2x20 ml), dried over anhydrous magnesium sulphate and evaporated in vacuo to afford a yellow oil. Purification by flash

chromatography [(MeOH:$CHCl_3$)(1:15) v/v)] afforded the title compound as a white solid (0.4g).

δ ($CDCl_3$): 0.96 (6H,t), 1.05 (3H,t), 1.2-2.4 (13H,m), 2.73 (3H,d), 3.18 (2H,m), 3.69 (1H,m), 3.82 (2H,d), 4.38 (1H,q), 4.88-5.03 (4H,m), 5.08 (2H,s), 6.35 (1H,t), 6.95 (1H,t), 7.08 (1H,q), 7.22-7.4 (15H,m), 7.51 (1H,d).

Description 10

N-tert-Butoxycarbonyl-(S)-aspartic acid methylamide (D10)



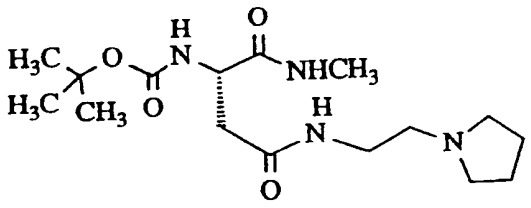
To a solution of N-tert-butoxycarbonyl-(S)-aspartic acid β-benzyl ester (66g) in dry tetrahydrofuran (100 ml) at -10°C was added diisopropylethylamine (38 ml) followed by ethyl chloroformate (23 ml) and a solution of methylamine (10g) in dry tetrahydrofuran (30 ml) was added. After 0.5h the reaction mixture was evaporated to dryness in vacuo and the residue, in ethyl acetate, was washed with 10% sodium carbonate, citric acid and water, and dried (Na_2SO_4). Evaporation to dryness in vacuo, followed by trituration with ethyl acetate-ether (1:1) afforded a solid (45g) that was hydrogenated in ethanol (600 ml) over 10% palladium on carbon (8g) until uptake of hydrogen ceased. The reaction mixture was filtered, and the

filtrate was evaporated to dryness to afford the title compound (D10) as a white solid (33.5g), m.p. 160-162°C (dec).

Found: C,48.69; H,7.35; N,11.12%. $C_{10}H_{18}N_2O_5$ requires C,48.77; H,7.37; N,11.38%.

Description 11

N-tert-Butoxycarbonyl-β-[(2-pyrrolidinoethyl)amide]-(S)-aspartic acid methylamide (D11)

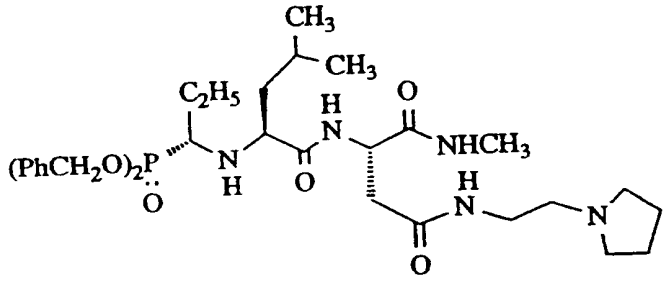


To a solution of N-tert-butoxycarbonyl-(S)-aspartic acid methylamide (D10) (5g) in dichloromethane (50 ml) at 0°C was added 1-(3-dimethylaminopropyl)-3- ethylcarbodiimide hydrochloride (4.74g) and 1-hydroxybenzotriazole (3.34g). After 10 mins N-(2-aminoethyl)pyrrolidine (2.82g) was added dropwise, and the solution was stirred at 0°C for 2h and then at room temperature overnight. The reaction mixture was washed with sodium bicarbonate solution, water, and dried (Na_2SO_4), and then evaporated to dryness in vacuo to give the title compound (D11) (1.0g).

δ ($CDCl_3$): 1.42(9H,s), 1.9(4H,m), 2.5-2.9(8H,m), 2.82(3H,d,J=5Hz), 3.38(2H,q,J=5Hz), 4.5(1H,m), 6.21(1H,m), 6.75(1H,m) and 7.02(1H,m).

Description 12

N-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-β-[(2-pyrrolidinoethyl)amide]-(S)-aspartic acid methylamide (D12)

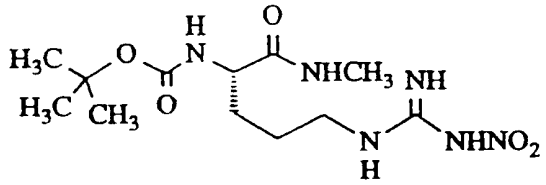


This compound was prepared from N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) and β-[(2-pyrrolidinoethyl)amide]-(S)-aspartic acid methylamide (prepared from (D11) by reaction with trifluoroacetic acid by the procedure described for (D5)) following the method of Description 11, m.p. 110-118°C (48% yield).

δ ($CDCl_3$): 0.85(6H,t,J=5Hz), 1.05(3H,t,J=7Hz), 1.4(3H,m), 1.6(3H,m), 1.85(approx. 6H,m), 2.6-2.85(approx. 10H,m), 3.3(1H,m), 3.45(1H,m), 3.65(1H,m), 4.85(1H,m), 5.0(4H,m), 7.45(10H,m), 7.5(1H,m) and 8.2(1H,d,J=7Hz).

Description 13

N^{α}-tert-Butoxycarbonyl-N^{ω}-nitro-(S)-arginine methylamide (D13)

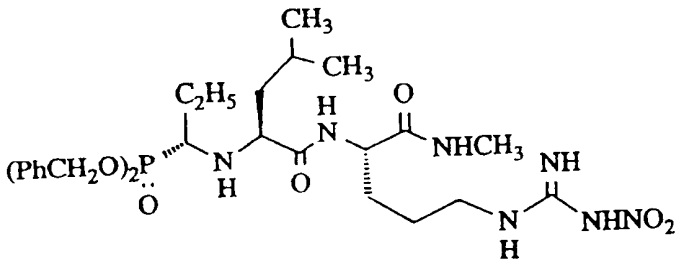


N^{α}-tert-Butoxycarbonyl-N^{ω}-nitro-(S)-arginine (3g, 9.4 mmol), 1-hydroxybenzotriazole (2.88g, 18.8 mmol) and methylamine hydrochloride (1.26g, 18.7 mmol) were dissolved in dry dimethylformamide (50 ml) and cooled in an ice-salt bath to -10°C. Diisopropylethylamine (3.3 ml, 18.78 mmol) was added followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.76g, 14.4 mmol). After 1h, at 0°C the reaction mixture was stirred for 18h at room temperature when t.l.c. [($CHCl_3$:MeOH:AcOH) (10:2:1) v/v] showed the reaction to be complete. After evaporation to dryness the solid was dissolved in water and applied to a column of Dowex 50W-X8 (ammonium form). This was eluted with water and eluant containing UV absorbing material was collected, concentrated and then shaken with Amberlite IRA-401 (acetate form) for 1h. The resin was collected and washed with water. The filtrate and washings were combined and lyophilised to give the product (D13) as a chromatographically pure white solid (2.6g, 84%).

Observed FAB $(M+H)^+$ 333. $C_{18}H_{24}N_6O_5$ requires M 332.

Description 14

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ω}-nitro-(S)-arginine methylamide (D14)

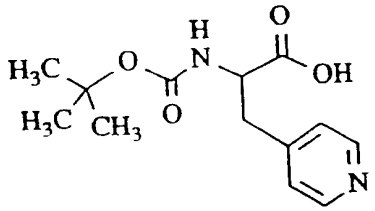


N^{α}-tert-Butoxycarbonyl-N^{ω}-nitro-(S)-arginine methylamide (D13) (0.142g, 0.43 mmol) was treated with 95% trifluoroacetic acid in water (5 ml) for 0.5h at 0°C. Excess acid was removed under reduced pressure and the residue twice evaporated with dry toluene. The residue was dissolved in dry dimethylformamide (2 ml) and the pH adjusted to 8-9 by the addition of diisopropylethylamine. This was added to a solution of N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (0.15g, 0.35 mmol) and 1-hydroxybenzotriazole (0.095g, 0.62 mmol) in dimethylformamide (5 ml). The mixture was cooled to -10 to -15°C and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.08g, 0.42 mmol) added. The reaction mixture was allowed to warm to room temperature and stirred for 18h. The mixture was then evaporated to dryness and purified by silica gel chromatography [(MeOH:$CHCl_3$) (1:9) v/v]. The product (D14) was obtained as a pale yellow foam. (0.16g, 71%).

Observed FAB $(M+H)^+$ 648. $C_{30}H_{46}N_7O_7P$ requires M 647.

Description 15

N^{α}-tert-Butoxycarbonyl-(R)-β-(4-pyridyl)alanine (D15A) and N^{α}-tert-butoxycarbonyl-(S)-β-(4-pyridyl)alanine (D15B)

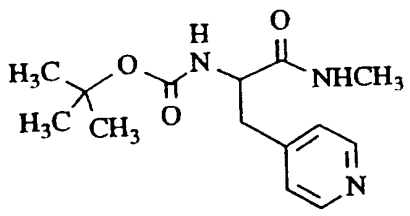


Crude racemic β-(4-pyridyl)alanine dihydrochloride[1] (0.88g, 3.66 mmol) was dissolved in water (30 ml) and solid $NaHCO_3$ (1.51g) added. When dissolved, dioxan (30 ml) was added and the mixture cooled in an ice-salt bath to 0°C. Di-tert-butyl dicarbonate (1.5g, 6.9 mmol) as a solution in dioxan (20 ml) was added and after 1h at 0-4°C, the mixture was stirred for 18h at room temperature. The reaction mixture was evaporated to dryness and a mixture of ethyl acetate, water and acetic acid (5:1:1, v/v) added. The precipitated solid was filtered off and the filtrate evaporated in vacuo. The residue was triturated with several portions of hot ethanol. When cool, the extracts were combined and evaporated to dryness. This process was repeated until the solid residue redissolved readily in ethanol. Evaporation of this solution gave the title compound (D15) as a racemic mixture, contaminated with sodium acetate. This was used without further purification.

[1]R.L. Bixler and C. Niemann, J. Org. Chem., 23, 575 (1958).

Description 16

N^{α}-tert-Butoxycarbonyl-(R)-β-(4-pyridyl)alanine methylamide (D16A) and N^{α}-tert-butoxycarbonyl-(S)-β-(4-pyridyl)alanine methylamide (D16B)

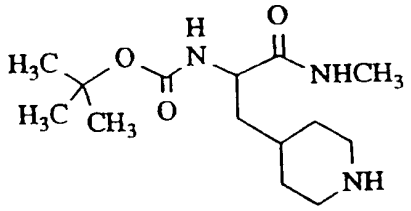


Crude racemic derivative (D15) (3.66 mmol) was dissolved in dry dimethylformamide (30 ml) and 1-hydroxybenzotriazole (1.13g, 7.38 mmol) and methylamine hydrochloride (0.5g, 7.40 mmol) added. The mixture was cooled to -10°C in an ice-salt bath and diisopropylethylamine (1.3 ml, 7.5 mmol) added. After 5 minutes, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.7g, 8.9 mmol) was added and after stirring for 1h at -5°C, the mixture was stirred overnight at room temperature. After evaporation to dryness, dichloromethane (50 ml) was added and then extracted with several portions (3x15 ml) of sat. aq. $NaHCO_3$. The organic phase was dried over anhydrous magnesium sulphate, filtered and the solvent removed in vacuo. The residue was purified by silica gel chromatography [(MeOH:$CHCl_3$) (1:9) v/v] to give the racemic title compound (D16) as a white solid (0.91g).

Observed FAB $(M+H)^+$ 280. $C_{14}H_{21}N_3O_3$ requires M 279.

Description 17

N^{α}-tert-Butoxycarbonyl-(R)-β-(4-piperidyl)alanine methylamide (D17A) and N^{α}-tert-butoxycarbonyl-(S)-β-(4-piperidyl)alanine methylamide (D17B)

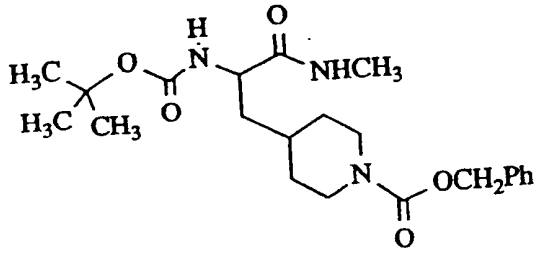


The mixture of isomers of amide (D16) (0.5g, 1.79 mmol) was dissolved in glacial acetic acid (45 ml) and degassed

under reduced pressure. A suspension of Adam's catalyst (0.2g) in glacial acetic acid (5 ml) was added and the mixture hydrogenated for 22h at atmospheric pressure. The reaction mixture was filtered through Kieselguhr and the solvent removed in vacuo to give the racemic product (D17) as a chromatographically pure oil (0.51g).

Description 18

N^{α}-tert-Butoxycarbonyl-(R)-β-(4-(N-benzyloxycarbonyl)-piperidyl)alanine methylamide (D18A) and N^{α}-tert-butoxy-carbonyl-(S)-β-(4-(N-benzyloxycarbonyl)piperidyl)alanine methylamide (D18B)



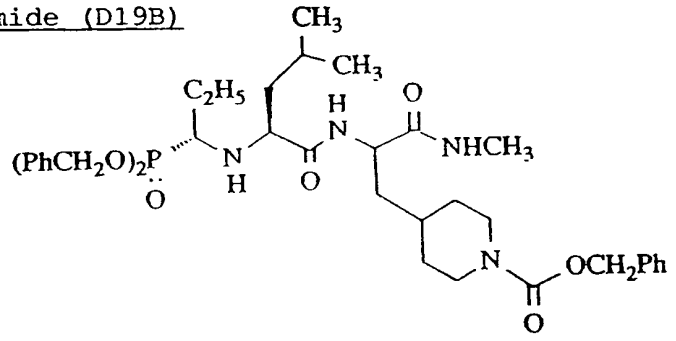
Isomer mixture (D17) (0.51g, 1.79 mmol) was dissolved in a dioxan/water mixture (2:1, 30 ml) and the pH adjusted to 7 by the addition of solid $NaHCO_3$. The mixture was cooled to 0°C, a second portion of $NaHCO_3$ (0.17g, 2 mmol) added followed by the portionwise addition of benzylchloroformate (0.3 ml, 2.1 mmol). After 1h at 0°C, the mixture was stirred overnight at room temperature. After evaporation to dryness, the residue was taken up in ethyl acetate and extracted with 10% aq. citric acid (2x), sat. aq. $NaHCO_3$ (2x) and sat. aq. NaCl. The organic phase was dried (anhydrous $MgSO_4$), filtered and the solvent removed in vacuo. The resultant oil was purified by

silica gel chromatography [(CH_2Cl_2:MeOH)(19:1)v/v] to give the racemic title compound (D18) as an oil which crystallised on standing under 60-80 light petroleum (0.68g, 91%), m.p. 130-131°C.

Observed FAB $(M+H)^+$ 420. $C_{22}H_{33}N_3O_5$ requires M 419.

Description 19

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(R)-β-(4-(N-benzyloxycarbonyl)piperidyl)alanine methylamide (D19A) and N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-β-(4-(N-benzyloxycarbonyl)piperidyl)alanine methylamide (D19B)

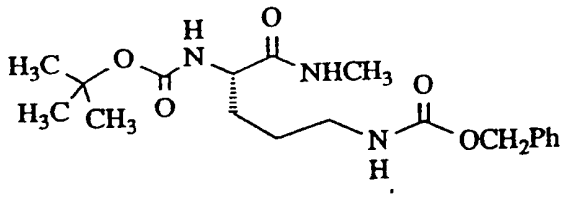


The protected amide isomer mixture (D18) (0.19g, 0.45 mmol) was treated with 95% trifluoroacetic acid in water (5 ml) for 0.5h at 0°C. Excess acid was removed under reduced pressure and the oily residue evaporated twice with dry toluene. The residue was dissolved in dry dimethylformamide (2 ml) and the pH adjusted to 8-9 by the addition of diisopropylethylamine. This was added to a solution of N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (0.15g, 0.35 mmol) and 1-hydroxybenzotriazole (0.11g, 0.7 mmol) in dimethylformamide (5 ml). The mixture was cooled to -10°C and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

(0.1g, 0.52 mmol) added. The mixture was allowed to warm to room temperature and then stirred for 18h. The reaction mixture was evaporated to dryness in vacuo and the residue redissolved in dichloromethane. This was extracted with 10% aq. citric acid, sat. aq. $NaHCO_3$ (2x) sat. aq. NaCl and then dried over anhydrous magnesium sulphate. Evaporation in vacuo gave the title compound isomer mixture (D19) as an oil which was purified by silica gel chromatography [$(CH_2Cl_2$:MeOH) (9:1)].

Description 20

N^{α}-tert-Butoxycarbonyl-N^{ε}-benzyloxycarbonyl-(S)-ornithine methylamide (D20)

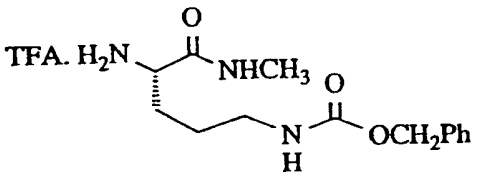


A solution of N^{α}-tert-butoxycarbonyl-N^{ε}-benzyloxycarbonyl-(S)-ornithine (1.5g, 0.004 mol) in anhydrous dichloromethane (60 ml) maintained at 0°C was sequentially treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.94g) and 1-hydroxybenzotriazole (0.66g) and then left stirring for 0.5h. Methylamine (excess) was bubbled through, flushed with nitrogen and the solution extracted with dilute citric acid (40 ml) and brine (40 ml). The organic phase was dried and evaporated to yield a viscous oil. Purification by flash chromatography [2% methanol in ethyl acetate] afforded the title compound (D20) as a white solid (1.2g).

Observed FAB $(M+H)^+$ 380. $C_{19}H_{29}N_3O_5$ requires M 379.

Description 21

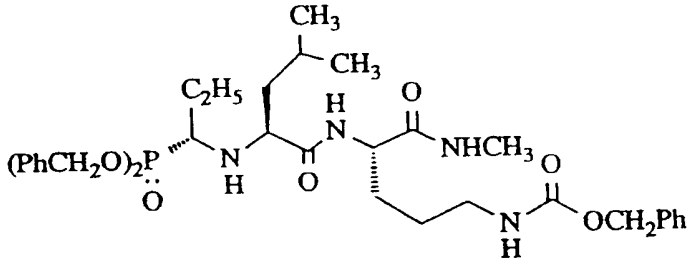
N^{α}-Benzyloxycarbonyl-(S)-ornithine methylamide, trifluoroacetate salt (D21)



A solution of the amide (D20) (0.14g) in dichloromethane (5 ml) maintained at 0°C was treated with trifluoroacetic acid (2 ml). The solution was left stirring at room temperature for 2h, then solvent evaporated in vacuo to afford crude title compound (D21) as an oil. This was used as such without further purification.

Description 22

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-ornithine methylamide (D22)

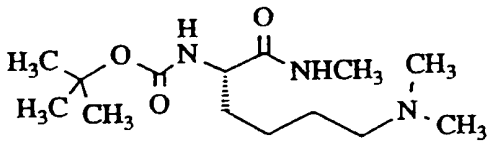


A stirred solution of N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (0.142g) in anhydrous dichloromethane (15 ml) maintained at 0°C was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.07g) and 1-hydroxybenzotriazole (0.049g). After 0.5h, the mixture was sequentially treated with crude salt (D21) and N,N-diisopropylethylamine (0.095g) and then left stirring at room temperature for 18h. The solution was then washed with 1M aq. citric acid (10 ml), sat. aq. $NaHCO_3$ (10 ml) and sat. aq. NaCl (10 ml). The organic fraction was dried and evaporated in vacuo to yield an oil. Purification by flash chromatography (5% methanol in chloroform) afforded the title compound (D22) as a white foam (0.095g).

Observed FAB $(M+H)^+$ 695. $C_{37}H_{51}N_4O_7P$ requires M 694.

Description 23

N^{α}-tert-Butoxycarbonyl-N^{ε}-dimethyl-(S)-lysine methylamide (D23)



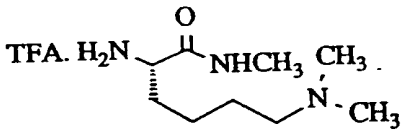
A solution of the amide (D4) (0.8g) in methanol (100 ml) was treated with 5% palladium on charcoal (1g). The suspension was diluted with 38% aqueous formaldehyde solution (6 ml) and hydrogenated at atmospheric pressure and ambient temperature for 48h. A further aliquot of catalyst (0.5g) and formaldehyde (3 ml) was added and

hydrogenation continued for a further 24h. The solution was filtered through Kieselguhr, solvent evaporated in vacuo and the residue purified by flash chromatography [($CHCl_3$:MeOH:NH_3)(12:2:1) v/v] to afford the title compound (D23) (0.29g).

Observed M^+ 287. $C_{14}H_{29}N_3O_3$ requires M 287.

Description 24

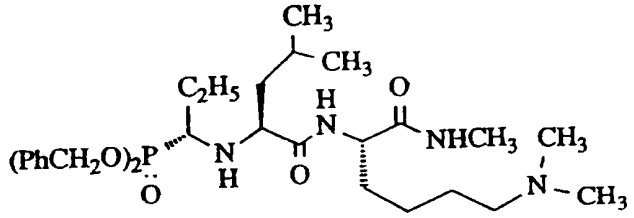
N^{ε}-Dimethyl-(S)-lysine methylamide, trifluoroacetate salt (D24)



A stirred solution of the amine (D23) (0.11g) maintained at 0°C in dichloromethane (5 ml) was treated with trifluoroacetic acid (2 ml). The solution was stirred for 1h, then solvent evaporated at reduced pressure to afford crude title compound (D24) as a clear oil. This was used as such without further purification.

Description 25

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-lysine methylamide (D25)

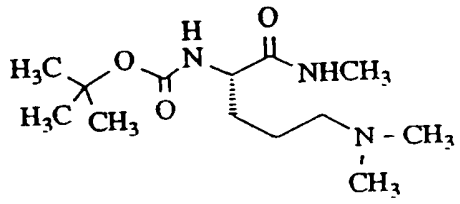


A stirred solution of N-(1-(R)-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (0.132g) in anhydrous dichloromethane (20 ml) was treated sequentially with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.066g) and 1-hydroxybenzotriazole (0.042g). The mixture was left stirring for 0.5h, treated with amine salt (D24) and N,N-diisopropylethylamine (0.148g) and stirring continued for 4h at room temperature. The mixture was washed with water (2x10 ml), sat. aq. $NaHCO_3$ (20 ml), dried over anhydrous magnesium sulphate and evaporated in vacuo to give a pale yellow oil. Purification by flash chromatography [$(CHCl_3$:MeOH:$NH_3)$(15:2:0.5)v/v] afforded the title compound (D25) as a white foam (0.11g).

Observed FAB $(M+H)^+$ 603. $C_{32}H_{51}N_4O_5P$ requires M 602.

Description 26

N^{α}-tert-Butoxycarbonyl-N^{ε}-dimethyl-(S)-ornithine methylamide (D26)

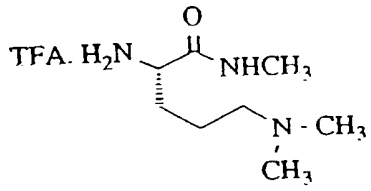


The title compound (D26) was prepared following the procedure described for the synthesis of N^{α}-tert-butoxycarbonyl-N^{ε}-dimethyl-(S)-lysine (D23) (yield: 69%).

Observed FAB $(M+H)^+$ 274. $C_{13}H_{27}N_3O_3$ requires M 273.

Description 27

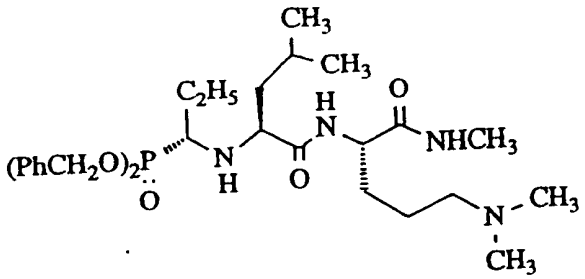
N^{ε}-Dimethyl-(S)-ornithine methylamide, trifluoroacetate salt (D27)



The title compound (D27) was prepared following the procedure described for the synthesis of N^{ε}-dimethyl-(S)-lysine methylamide, trifluoroacetate salt (D24). The title compound (D27) was used without any formal purification.

Description 28

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-ornithine methylamide (D28)

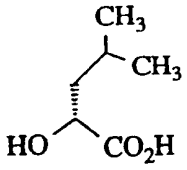


The title compound (D28) was prepared following the procedure described for the synthesis of the dibenzyl ester (D25). (Yield: 56%).

Observed FAB $(M+H)^+$ 589. $C_{31}H_{49}N_4O_5P$ requires M 588.

Description 29

(R)-2-Hydroxy-4-methylpentanoic acid (D29)



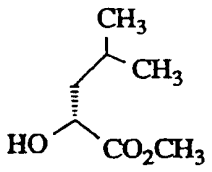
The title compound was prepared by modification of the method of G. Iwasaki et al, Chem. Pharm. Bull. 1989, 37(2), 280. A solution of sodium nitrite (33.8g) in water (100 ml) was added dropwise over 1.75h to a stirred

solution of D-leucine (20g, 0.15 mol) in 3N sulphuric acid (700 ml) and 50% aqueous acetic acid (500 ml) at 92-98°C. The mixture was heated for a further 3h at 98-99°C, then cooled and extracted with diethyl ether (4x250 ml). The combined organic layers were dried with anhydrous magnesium sulphate, filtered and evaporated to dryness to give the title compound (D29) as a pale yellow solid (18.91g, 94%). A sample was recrystallized from $CHCl_3$/pentane as white needles, m.p. 77.5-79°C.

$[\alpha]^{22}_D$ = 26.41 (c=0.98 1N NaOH).

Description 30

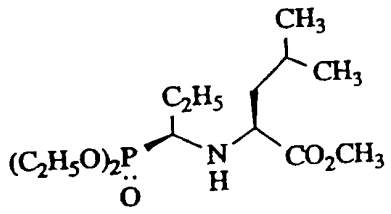
Methyl (R)-2-hydroxy-4-methylpentanoate (D30)



A mixture of acid (D29) (3.97g, 0.03 mol) in methanol (100 ml) and 1N ethereal HCl (10 ml) was maintained at ambient temperature for 5 days. After evaporation to dryness and purification by chromatography on silica gel with diethyl ether as eluant the title compound (D30) was obtained as a pale yellow oil (3.51g, 80%) and was carried forward without further purification.

Description 31

N-(1-(S)-Diethoxyphosphinylpropyl)-(S)-leucine methyl ester (D31)



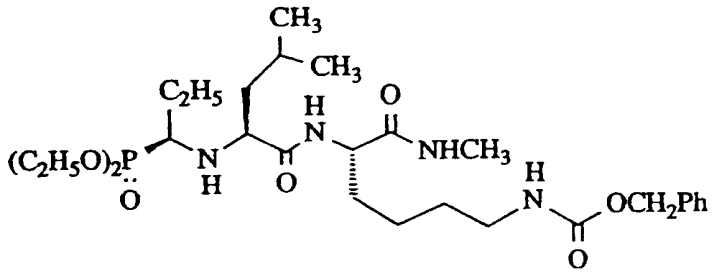
The alcohol (D31) (0.696g, 0.0048 mol) in dichloromethane (10 ml) was cooled to -60°C and then 2,6-lutidine (0.68 ml) followed by trifluoromethanesulphonic anhydride (0.92 ml) were added. After allowing to warm to 0°C a solution of (S)-1-aminopropylphosphonic acid, diethyl ester[1] (0.93g, 0.0048 mol) in dichloromethane (10 ml) was added, followed by proton sponge (1.02g). The mixture was stirred for 4 days in the dark under N_2 and then filtered, the solid washed with chloroform and the filtrate washed with 10% citric acid (x2) followed by water. The organic layer was dried with anhydrous magnesium sulphate, filtered and evaporated to give the crude product as a red oil. Purification by column chromatography (first silica gel with gradient elution 0-6% MeOH/$CHCl_3$, then silica gel with gradient elution 0-2% MeOH/EtOAC) gave the title compound (D31) as a pale yellow oil (0.48g, 33%).

Observed M^+ 323.1862. $C_{14}H_{30}NO_5P$ requires 323.1858. Other physical properties in accord with the literature[1]

[1] EP-A-0 401 963

Description 32

N^{α}-[N-((S)-Diethoxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methyl amide (D32)



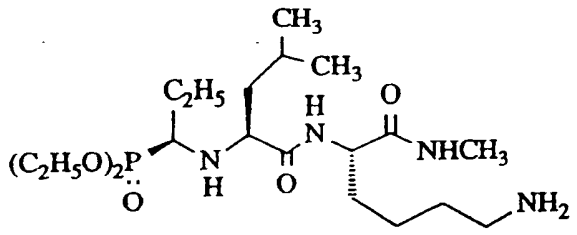
N-(1-(S)-Diethoxyphosphinylpropyl)-(S)-leucine[1] was prepared from the corresponding methyl ester (D31) by standard base hydrolysis. A solution of this acid (0.25g, 0.00081 mol) in dichloromethane (9 ml) was cooled to 0°C under N_2 and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.17g) then 1-hydroxybenzotriazole (0.12g) were added and the mixture stirred for 1h at this temperature. N^{ε}-Benzyloxycarbonyl-(S)-lysine methylamide trifluoroacetate (D5) (from 0.0097 mol of the corresponding N^{α}-tert-butoxycarbonyl derivative (D4)) in dichloromethane (5 ml) was added followed by diisopropylethylamine (0.35 ml) and the mixture stirred at room temperature overnight. After dilution with chloroform (20 ml) the mixture was washed with 10% citric acid (x2) and water. The organic layer was dried (anhydrous magnesium sulphate) and volatile material evaporated in vacuo to give a colourless gum. Purification by chromatography on silica gel (gradient elution 0-5% MeOH/EtOAc) gave the product (D32) as a colourless gum (0.36g, 76%).

Observed M^+ 584.3346. $C_{28}H_{49}N_4O_7P$ requires 584.3339.

[1] EP-A-0 401 963

Description 33

N^{α}-[N-((S)-1-Diethoxyphosphinylpropyl)-(S)-leucyl]-(S)-lysine methylamide (D33)

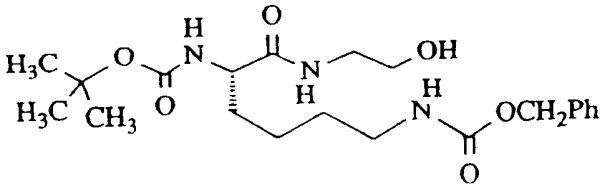


N^{α}-[N-((S)-1-Diethoxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methylamide (D32) (0.18g, 0.0003 mol) in methanol (25 ml) with 10% palladium on charcoal (0.2g) was hydrogenated at atmospheric pressure overnight. After filtration through Kieselguhr and evaporation to dryness the title compound (D33) was obtained as a clear gum.

Observed M^+ 450.2982. $C_{20}H_{43}N_4O_5P$ requires 450.2972.

Description 34

N^{ε}-Benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-(S)-lysine 2-hydroxyethylamide (D34)

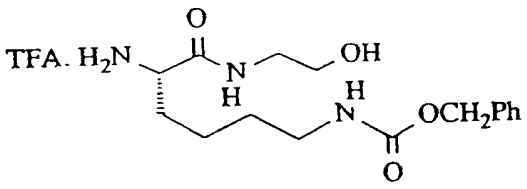


A solution of N^{ε}-benzyloxycarbonyl-N^{α}-tert-butoxycarbonyl-(S)-lysine (5g, 0.0132 mol) in dichloromethane (50 ml) was cooled to 0°C and 1-hydroxybenzotriazole (1.78g) followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.52g) were added. The mixture was stirred at 0°C for 1h and then ethanolamine (0.88g, 0.0144 mol) added in one portion. The solution was stirred overnight at room temperature, washed with water (2x30 ml), saturated sodium bicarbonate (30 ml) and finally water (30 ml). The organic layer was dried with anhydrous magnesium sulphate, filtered and evaporated to dryness to give the product (D34) as a clear gum (5.44g, 97%) which was used without further purification.

Observed M^+ 423.2365. $C_{21}H_{33}N_3O_6$ requires 423.2369.

Description 35

N^{ε}-Benzyloxycarbonyl-(S)-lysine 2-hydroxyethylamide, trifluoroacetate salt (D35)



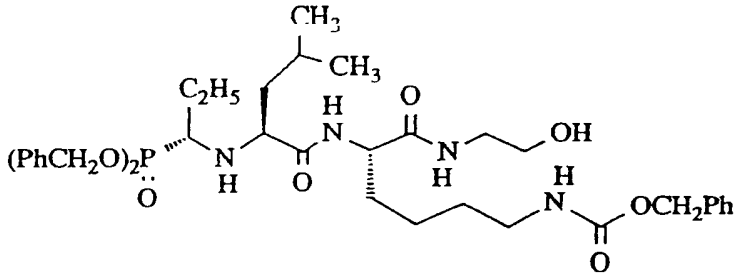
Trifluoroacetic acid (30 ml) was added to a stirred solution of the lysine derivative (D34) (2.64g, 0.0062 mol) in dichloromethane (50 ml) at 0°C. After 3h stirring at ice-bath temperature volatile material was removed

under reduced pressure to give the crude product (D35) in quantitative yield. This was used in subsequent steps without further purification.

Observed FAB $(M+H)^+$ 324. $C_{16}H_{25}N_3O_4$ requires M 323.

Description 36

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine 2-hydroxyethylamide (D36)



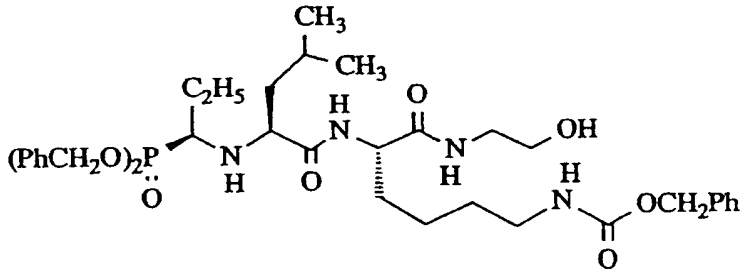
A solution of N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3A) (1.61g, 0.0037 mol) in dichloromethane (60 ml) was cooled to 0°C and 1-hydroxybenzotriazole (0.78g, 0.0058 mol) was added followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.09g, 0.0057 mol). The mixture was stirred for 1h at ice-bath temperature and then a solution of the trifluoroacetate salt (D35) (1.2 equivalents) in dichloromethane (15 ml) was added followed by diisopropylethylamine (3.36g) to ensure neutralization of excess trifluoroacetic acid. After stirring overnight at room temperature the reaction mixture was washed with 10% aqueous citric acid (2x20 ml), water (20 ml), saturated sodium bicarbonate (2x20 ml) and finally water. The organic layer was dried (anhydrous magnesium sulphate) filtered and evaporated to dryness. The residue was chromatographed on silica gel with 1:1

increasing percentage of methanol (to 10%). Further purification on silica gel with a gradient of 0-4% MeOH/$CHCl_3$ gave the title compound (D36) as a clear gum (0.83g, 30%).

Observed M^+ 738. $C_{39}H_{55}N_4O_8P$ requires M 738.

Description 37

N^{α}-[N-((S)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine 2-hydroxyethylamide (D37)

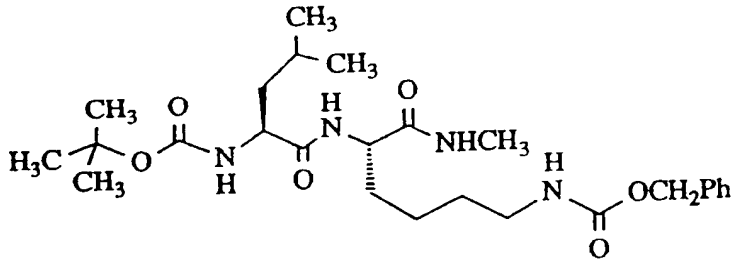


The title compound (D37) (1.035g) was prepared from N-((S)-1-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3B) (0.67g, 0.0015 mol) and the trifluoroacetate salt (D35) by the method given in Description 36.

Observed FAB $(M+H)^+$ 739. $C_{39}H_{55}N_4O_8P$ requires M 738.

Description 38

N^{α}-[(S)-tert-Butoxycarbonyl-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methylamide (D38)



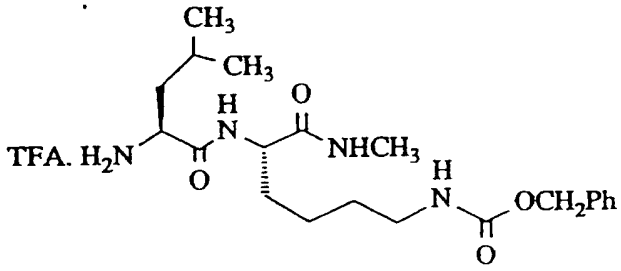
N-tert-Butyloxycarbonyl-(S)-leucine (25g, 0.11 mol) was dissolved in dichloromethane (250 ml) and cooled to 0°C. 1,1'-Carbonyldiimidazole (18g, 0.113 mol) was added and the mixture left stirring at 0°C for 0.5h. The solution was allowed to warm to room temperature for 15 mins and then recooled to 0°C.

N^{ε}-Benzyloxycarbonyl-(S)-lysine methylamide trifluoroacetate salt (D5) (47g, 0.118 mol) in dichloromethane (150 ml) was added followed by the immediate addition of diisopropylethylamine (30.5g, 0.236 mol). The reaction mixture was left stirring overnight at room temperature and then treated with water and extracted with chloroform. The combined organic layers were washed with dilute hydrochloric acid (2x100 ml), water (2x100 ml), aqueous sodium carbonate (3x100 ml) and brine. The organic layer was dried with anhydrous magnesium sulphate and evaporated to dryness to give the title compound (D38) (50g, 93%) which was used without further purification.

Observed $(M+H)^+$ 507. $C_{26}H_{42}N_4O_6$ requires M 506.

Description 39

N^{α}-[(S)-Leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methylamide (D39)

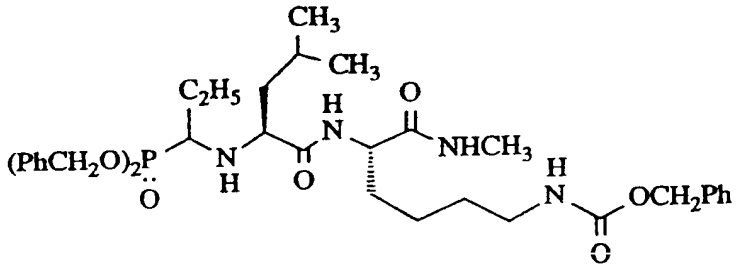


A cooled solution of the amide (D38) (50g, 0.099 mol) in dichloromethane (150 ml) was treated with trifluoroacetic acid (150 ml). After 3h the solvent was evaporated under reduced pressure. The resulting oil was dissolved in methanol and acidified with dilute hydrochloric acid to give the hydrochloride salt. Water was added and non-basic organic material extracted out with ethyl acetate (2x200 ml). The aqueous layer was basified to pH 8-9 with 10% sodium hydroxide and then extracted with ethyl acetate (3x150 ml). These combined organic extracts were dried (Na_2SO_4) and evaporated to dryness to give the title compound (D39) (20g, 50%) as a white solid.

Observed $(M+H)^+$ 407. $C_{21}H_{34}N_4O_4$ requires M 406.

Description 40

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methylamide and N^{α}-[N-((S)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine methylamide (D40)



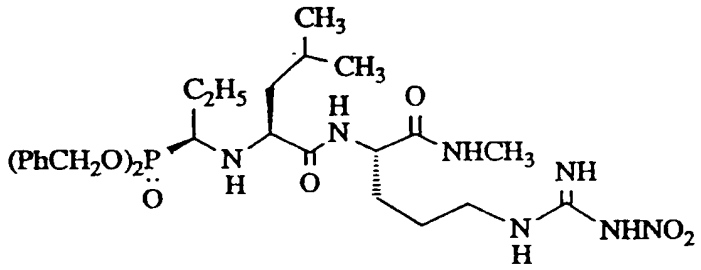
Dibenzyl ((1-trifluoromethanesulphonyloxy)propyl)-phosphonate (D2) (4.4g, 0.0098 mol) was dissolved in dry dichloromethane (20 ml). N^{α}-[(S)-Leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methylamide (D39) (4.0g, 0.0098 mol) and Proton Sponge (2.0g, 0.0098 mol) were added to the solution and the reaction mixture was stirred in the dark at room temperature for 10 days.

The solution was diluted further with chloroform, washed with 10% citric acid (2x50 ml) and water (3x50 ml), dried (anhydrous $MgSO_4$) and evaporated to dryness to give an orange oil. This was purified by column chromatography (silica gel, 2% MeOH/EtOAc) to give the mixture of title compounds (D40) (2.55g, 35%) as a clear oil.

Observed $(M+H)^+$ 709. $C_{38}H_{53}N_4O_7P$ requires M 708.

Description 41

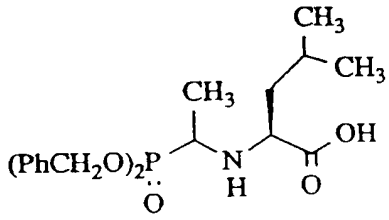
N^{α}-[N-((S)-1-Dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ω}-nitro-(S)-arginine methylamide (D41)



The title compound (D41) (0.72g, 74%) was prepared from N^{α}-tert-butoxycarbonyl-N^{ω}-nitro-(S)-arginine methylamide (D13) (0.6g, 0.0018 mol) and N-((S)-1-dibenzyloxyphosphinylpropyl)-(S)-leucine (D3B) (0.633g, 0.0014 mol) by the method described in Description 14 with the exception that dichloromethane was used as reaction solvent with sufficient dimethylformamide to effect solution.

Description 42

N-((R)-1-Dibenzyloxyphosphinylethyl)-(S)-leucine (D42A) and N-((S)-1-dibenzyloxyphosphinylethyl)-(S)-leucine (D42B)

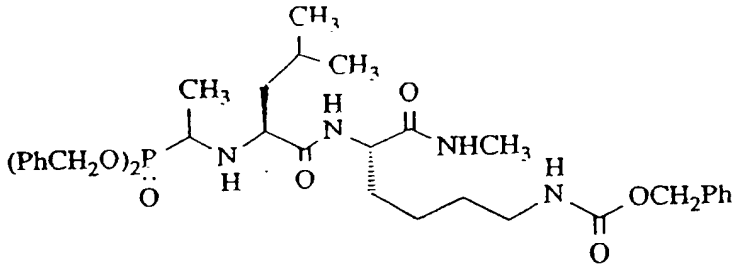


The title mixture of diastereoisomers (D42) was prepared analogously to the method in Description 3, Method B, as a white solid.

Observed FAB $(M+H)^+$ 420. $C_{22}H_{30}NO_5P$ requires M 419.

Description 43

N^{α}-[N-((R)-1-Dibenzyloxyphosphinylethyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine methylamide (D43A) and N^{α}-[N-((S)-1-Dibenzyloxyphosphinylethyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine methylamide (D43B)

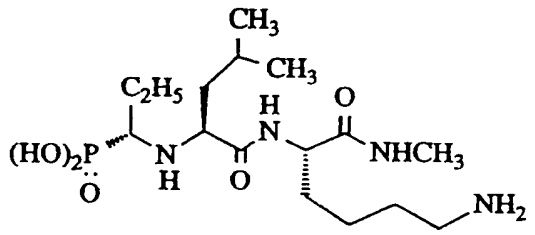


The title compound (D43) was obtained as a clear oil (1.0g, 63%) from the acid (D42) (1.0g, 0.002 mol) following the general method of Description 6.

Observed FAB $(M+H)^+$ 695. $C_{37}H_{51}N_4O_7P$ requires M 694.

Example 1

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide (E1)



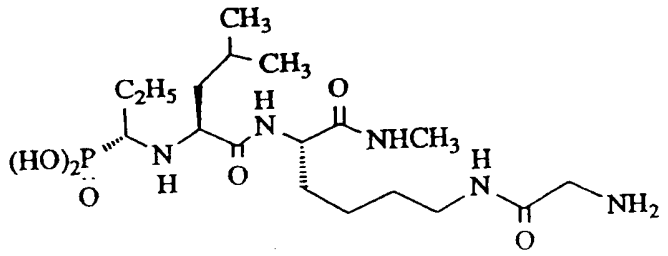
The dibenzyl ester (D6) (0.105g, 0.16 mmol) was dissolved in ethanol (40 ml) and hydrogenated over 10% palladium on charcoal at atmospheric pressure. The solution was filtered through Kieselguhr and solvent evaporated in vacuo to give the title compound (E1) (0.01g).

Observed FAB $(M+H)^+$ 395. $C_{16}H_{35}O_5N_4P$ requires M 394.

δ ($CDCl_3/CD_3OD$): 0.95 (6H,dd), 1.08 (3H,t), 1.4-2.0 (9H,m), 2.65 (1H,m), 2.75 (3H,s), 2.92 (2H,m), 3.45 (2H,m), 4.18 (1H,m), 4.4 (1H,m).

Example 2

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-N^{ε}-glycyl-(S)-lysine methylamide (E2)



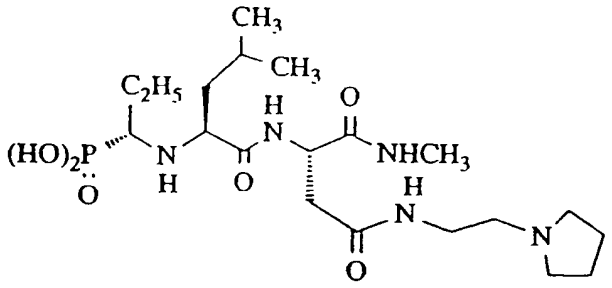
The dibenzyl ester (D9) (0.43g) was dissolved in ethanol (60 ml) and hydrogenated over 10% palladium on charcoal at atmospheric pressure. The solution was filtered through Kieselguhr and solvent evaporated in vacuo to give the title compound (E2) (0.059g).

Observed FAB $(M+H)^+$ 452. $C_{18}H_{38}O_6N_5P$ requires M 451.

δ (CD_3OD): 0.78-0.95 (9H,m), 1.19-1.9 (11H,m), 2.31 (1H,m), 2.62 (3H,s), 3.05-3.25 (2H,m), 3.30-3.65 (2H,m), 3.83-3.92 (1H,bt), 4.15-4.3 (1H,t).

Example 3

N-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-β-[(2-pyrrolidinoethyl)amide]-(S)-aspartic acid methylamide (E3)

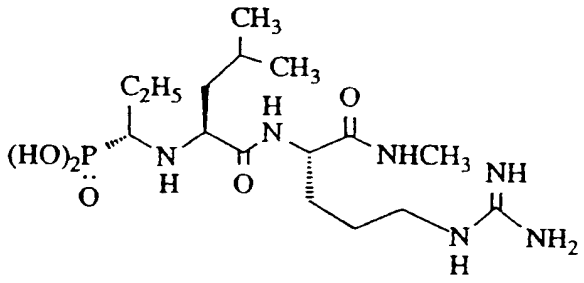


This compound was prepared from N-[N-[(R)-dibenzyloxy-phosphinylpropyl]-(S)-leucine-β-[(2-pyrrolidinoethyl)-amide]]-(S)-aspartic acid methylamide (D12) by hydrogenation over 10% palladium on charcoal at atmospheric pressure. m.p. 130-135°C (95% yield).

δ (CD_3OD): 1.0(6H,t,J=6Hz), 1.1(3H,t,J=7Hz), 1.55-1.8(4H,m), 2.0(1H,m), 2.15(4H,m), 2.6-2.85(6H,m), 3.3-3.75(approx. 8H,m), 4.1(1H,t,J=6Hz) and 4.75(1H,t,J=5Hz).

Example 4

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(S)-arginine methylamide (E4)



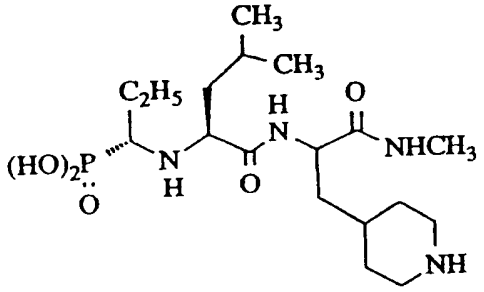
The fully protected derivative (D14) was dissolved in a mixture of glacial acetic acid (40 ml) and water (10 ml) and hydrogenated over 10% palladium on charcoal catalyst for 18h at atmospheric pressure. The solution was filtered through Kieselguhr and solvent evaporated in vacuo to give the title compound (E4) (0.085g).

Observed FAB $(M+H)^+$ 423. $C_{16}H_{35}N_6O_5P$ requires M 422.

δ (CD_3OD): 0.95(6H,dd), 1.03(3H,t), 1.5-1.94(9H,m), 2.56(1H,m), 2.72(3H,s), 3.17(2H,m), 4.02(1H,t), 4.37(1H,t).

Example 5

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(R)-β-(4-piperidyl)alanine methylamide (E5A) and
N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-β-(4-piperidyl)alanine methylamide (E5B)



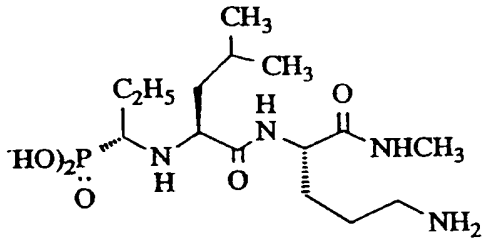
The fully protected derivative (D19) (0.24g) was dissolved in ethanol (50 ml) and hydrogenated over 10% palladium on charcoal at atmospheric pressure. The solution was filtered through Kieselguhr and solvent evaporated in vacuo. The residue was triturated with ether to remove slight impurities leaving the product (E5) as an off-white solid (0.13g).

Observed FAB $(M+H)^+$ 421. $C_{18}H_{37}N_4O_5P$ requires M 420.

δ (CD_3OD): 0.95(6H,dd), 1.02,1.05(3H, overlapping triplets), 1.4-2.0(12H,m), 2.4-2.55(1H, 2 overlapping m), 2.71(3H,d), 2.81-3.03(2H,m), 3.34(4H,br m), 3.68-3.81(1H, dt), 4.40(1H,m).

Example 6

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(S)-ornithine methylamide (E6)



A solution of phosphonic diester (D22) (0.07g) in methanol (20 ml) was treated with 5% palladium on charcoal. The suspension was hydrogenated at atmospheric pressure for 24h, filtered through Kieselguhr and solvent evaporated in vacuo. The residue was triturated with diethyl ether (5 ml) to give the title compound (E6) as a white solid (0.03g), m.p. 165-169°C.

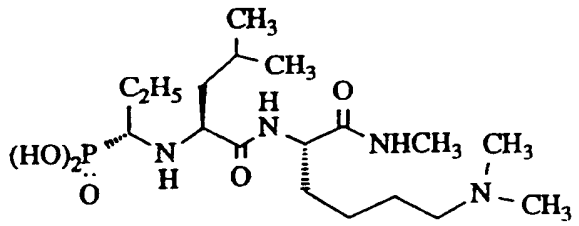
Observed FAB $(M+H)^+$ 381. $C_{15}H_{33}N_4O_5P$ requires M 380.

δ ($CDCl_3/CD_3OD$): 1.0(6H,dd), 1.12(3H,t), 1.6-1.9(8H,m), 2.05(1H,m), 2.7(1H,m), 2.75(3H,s), 2.9(2H,t), 4.42(1H,m), 4.55(1H,t).

The S,S,S diastereoisomer is prepared by a similar method.

Example 7

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-lysine methylamide (E7)



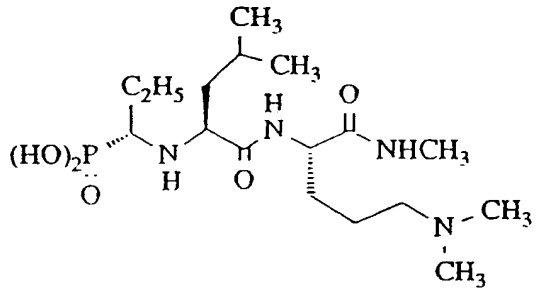
A solution of phosphonic diester (D25) (0.75g) in methanol (20 ml) was treated with 5% palladium on charcoal (0.05g) and hydrogenated at atmospheric pressure for 24h. The solution was filtered through Kieselguhr and solvent evaporated in vacuo. The residue was triturated with diethyl ether (10 ml) to give the title compound (E7) as white solid (0.045g).

Observed FAB $(M+H)^+$ 423. $C_{18}H_{39}N_4O_5P$ requires M 422.

δ (CD_3OD): 1.0(6H,dd), 1.1(3H,t), 1.3-1.85(10H,m), 2.0(1H,m), 2.6(1H,m), 2.75(3H,s), 2.8(6H,s), 3.05(2H,t), 4.35(2H,m).

Example 8

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-ornithine methylamide (E8)

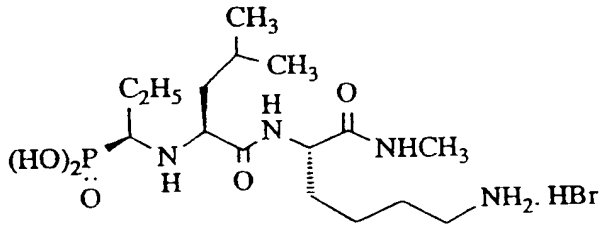


The title compound (E8) was prepared from the dibenzyl ester (D28) following the procedure described for the synthesis of N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-lysine methylamide (E7). (Yield: 74%). m.p. 88.5-90°C.

Observed FAB $(M+H)^+$ 409. $C_{17}H_{37}N_4O_5P$ requires M 408.

Example 9

N^{α}-[N-((S)-1-Phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide, hydrobromide salt (E9)



Bromotrimethylsilane (0.48 ml, 12 equiv.) was added to a solution of the diethyl ester (D33) (0.14g, 0.0024 mol) in dry acetonitrile (10 ml) and stirred at room temperature for 3 days.

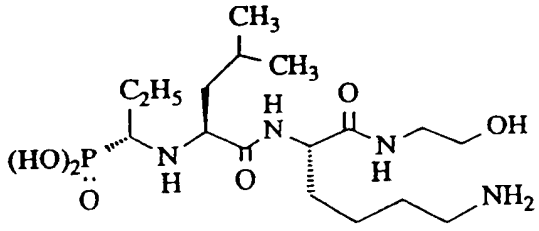
The resulting yellow solution was evaporated to dryness and treated with methanol/water. After evaporation and repeated treatment with methanol/water the product (E9) was obtained in quantitative yield as a pale orange foam.

Observed FAB $(M+H)^+$ 395. $C_{16}H_{35}N_4O_5P$ requires M 394.

δ (CD_3OD): 1.01(6H,dd), 1.18(3H,t), 1.3-2.15(11H,m), 2.74(3H,s), 2.98(2H,t), 3.19(1H,m), 4.3(1H,m), 4.42(1H,t).

Example 10

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(S)-lysine 2-hydroxyethylamide (E10)



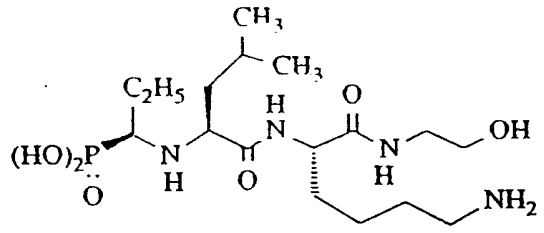
A solution of the dibenzyl ester (D36) (0.8g, 0.0011 mol) in methanol (100 ml) with 10% palladium on charcoal was hydrogenated at atmospheric pressure overnight. After filtration through Kieselguhr and evaporation to dryness the residue was taken up in doubly distilled water, refiltered and freeze-dried to give the title compound (E10) as a white foam (0.39g, 85%).

Observed FAB $(M+H)^+$ 425. $C_{17}H_{37}N_4O_6P$ requires M 424.

δ (CD_3OD): 0.98(6H,dd), 1.05(3H,t), 1.3-2.05(11H, overlapping m), 2.54(1H,br m), 2.92(2H,m), 3.3(2H,m, overlaps $C\underline{H}D_2OD$ solvent signal), 3.6(2H,t), 4.10(1H,br m), 4.38(1H,dd).

Example 11

N^{α}-[N-((S)-1-Phosphonopropyl)-(S)-leucyl]-(S)-lysine 2-hydroxyethylamide (E11)



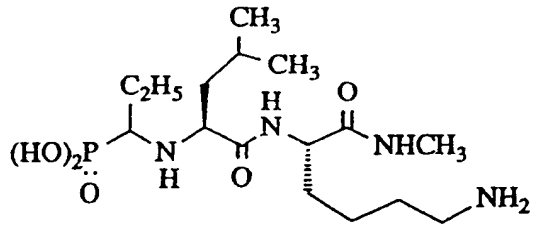
The title compound (E11) was obtained by atmospheric pressure hydrogenation of the dibenzyl ester (D37) by the method given in Example 10.

Observed FAB $(M+H)^+$ 425. $C_{17}H_{37}N_4O_6P$ requires M 424.

δ (CD_3OD): 0.97 (6H,dd), 1.12 (3H,t) 1.35-2.10 (11H, overlapping m), 2.46 (1H,m), 2.95 (2H,m), 3.32 (2H,m, overlaps $C\underline{H}D_2OD$ solvent signal), 3.53 (1H,m), 3.65 (2H,m), 4.49 (1H,m).

Example 12

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide and N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide (E12)

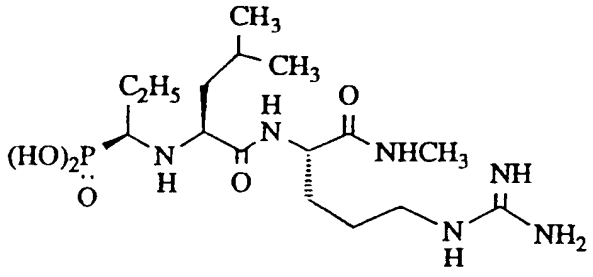


The mixture of dibenzyl esters (D40) (2.55 g, 0.004 mol) in ethanol (100 ml) was hydrogenated over 10% palladium on charcoal at atmospheric pressure. The solution was filtered through Kieselguhr and the solvent evaporated in vacuo to give the title compound (E12) as a white solid (1.0g, 71%).

Observed FAB $(M+H)^+$ 395. $C_{16}H_{35}N_4O_5P$ requires M 394.

Example 13

N^{α}-[N-((S)-1-Phosphonopropyl)-(S)-leucyl]-(S)-arginine methylamide (E13)



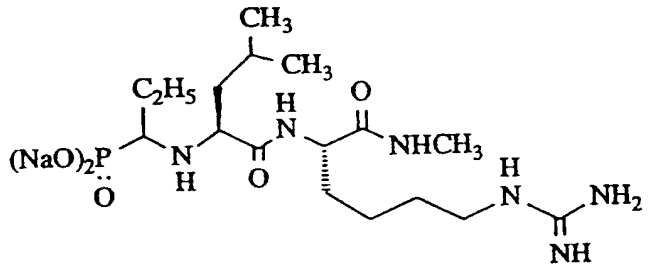
The title compound (E13) (0.5g, 90%) was prepared from the dibenzyl ester (D41) (0.633g, 0.014 mol) by the method of Example 4.

Observed FAB $(M+H)^+$ 423. $C_{16}H_{35}N_6O_5P$ requires M 422.

δ (CD_3OD): 0.97(6H,t), 1.10(3H,t), 1.49-1.95(9H,m), 2.50(1H,m), 3.21(2H,m), 3.60(1H,m), 4.94(1H,m).

Example 14

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(S)-homoarginine methylamide, disodium salt (E14A) and N^{α}-[N-((S)-1-phosphonopropyl-(S)-leucyl]-(S)-homoarginine methylamide, disodium salt (E14B)

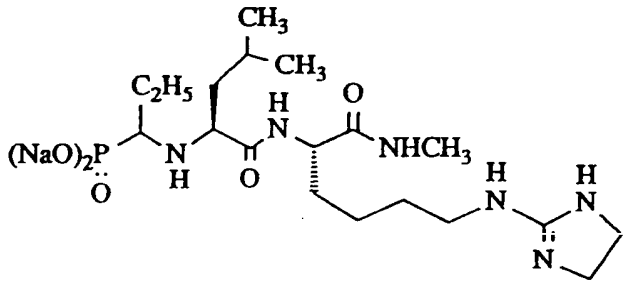


The mixture of R,S,S and S,S,S isomers (E12) (34.5mg, 0.087 mmol) in water (0.5 ml) was treated with sodium bicarbonate (44 mg, 6 equiv.) followed by 2-methyl-2-thiopseudourea sulphate (24.3 mg, 1 equiv.) and stirred at room temperature for 3h. Additional portions of sodium bicarbonate (8.5 mg) and 2-methyl-2-thiopseudourea (15 mg) were added and the mixture then heated at 70°C for 1.5h. Purification by reverse phase chromatography gave the title compound mixture of isomers (E14) as a white solid.

Observed FAB $(M+H)^+$ 481. $C_{17}H_{35}N_6O_5PNa_2$ requires M 480.

Example 15

N^{α}-[N-((R)-1-Phosphonopropyl)-(S)-leucyl]-(S)-N^{ε}-(2-imidazolinyl)-lysine methylamide, disodium salt (E15A) and N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-N^{ε}-(2-imidazolinyl)-lysine methylamide, disodium salt (E15B)

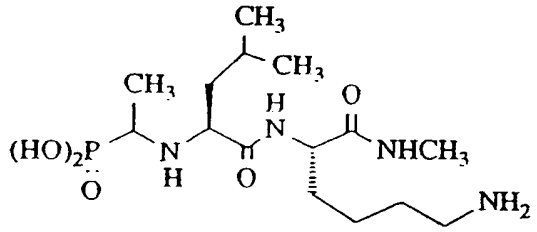


The title mixture of diastereoisomers (E15) was prepared from the phosphonic acid mixture of isomers (E12) (36.9 mg, 0.0935 mmol), 2-methylthio-2-imidazoline hydroiodide (45.6 mg and, after 3h, 13.9 mg) and sodium bicarbonate (47 mg and, after 3h, 7.8 mg) by the general method of Example 14.

Observed FAB $(M+H)^+$ 507. $C_{19}H_{37}N_6O_5PNa_2$ requires 506.

Example 16

N^{α}-[N-((R)-1-Phosphonoethyl)-(S)-leucyl]-(S)-lysine methylamide (E16A) and N^{α}-[N-((S)-1-phosphonoethyl)-(S)-leucyl]-(S)-lysine methylamide (E16B)



The dibenzyl ester (D43) (1.0.g, 0.0014 mol) was hydrogenated at atmospheric pressure by the method of Example 1 to give the title compound mixture (E16) in quantitative yield as a white crystalline solid.

Observed FAB $(M+H)^+$ 381. $C_{15}H_{33}N_4O_5P$ requires M 380.

COLLAGENASE INHIBITOR ASSAY

The test is performed essentially as in Cawston and Barrett, Anal. Biochem. 99, 340-345 (1979). Compounds for testing are dissolved in methanol by sonication and added to collagenase (purified from culture supernatants from the human lung fibroblast cell line, WI-38) in buffer. After a 5 min pre-incubation at 37°C, the assay tubes are cooled to 4°C and ^{3}H-acetylated rat skin type I collagen is added. The assay tubes are incubated at 37°C overnight. The ^{3}H-collagen forms insoluble fibrils, which are the substrate for the enzyme.

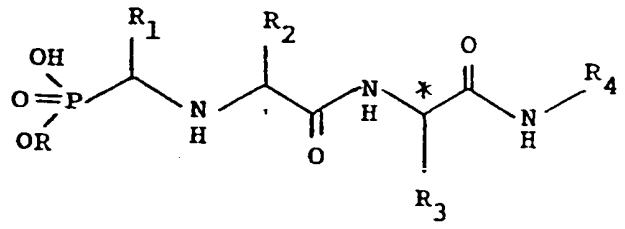
To terminate the assay, the assay tubes are spun at 12000 rpm for 15 minutes. Undigested ^{3}H-collagen is pelleted, while digested ^{3}H-collagen is found as soluble peptides in the supernatant. A sample of the supernatant is taken for liquid scintillation counting.

The activity of collagenase inhibitors (IC_{50}: 50% inhibitory concentration) is expressed as that concentration of compound that inhibits a known (standard) concentration of enzyme by 50%.

The compounds of Examples E1-E9 had IC_{50} values between 1.8×10^{-7} and 2.2×10^{-5}M.

Claims:

1. A compound of the formula (I) or a pharmaceutically acceptable salt, solvate or hydrate thereof:



(I)

in which,

R is hydrogen, C_{1-6} alkyl or optionally substituted benzyl;

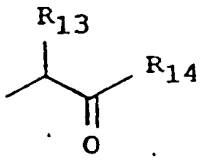
R_1 is hydrogen or C_{1-6} alkyl;

R_2 is C_{3-6} alkyl;

R_3 is $-(CH_2)_nNR_5R_6$, $-(CH_2)_nNHCOR_7$, $-(CH_2)_nCONH(CH_2)_qNR_5R_6$, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ or $-(CH_2)_n-R_{10}$ where n is an integer from 1 to 6 and each of R_5 and R_6 is independently hydrogen or alkyl, or R_5 and R_6 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring with an optional oxygen or sulphur atom or an optionally substituted second nitrogen atom in the ring, R_7 is alkyl or $-(CH_2)_nNR_5R_6$, R_8 is hydrogen or alkyl, R_9 is hydrogen or alkyl or R_9 and R_5 together with the nitrogen atoms to which they are bonded form an optionally substituted 5-, 6- or 7-membered ring, and R_{10} is an optionally substituted piperidyl ring;

m is 1 or 2, and q is 2 to 4; and

R_4 is hydrogen, alkyl, and $-CH_2-(CH_2)_nOR_{11}$ or $-CH_2-(CH_2)_nOCOR_{12}$ or



where n is an integer from 1 to 6; R_{11}, R_{12} and R_{13} are hydrogen or C_{1-6}alkyl; and R_{14} is hydroxy or $-O-C_{1-6}$alkyl or $-NR_5R_6$ (where R_5 and R_6 may be linked to form a heterocyclic ring).

2. A compound according to claim 1 in which R is hydrogen, methyl or ethyl.

3. A compound according to either of claims 1 or 2 in which R_1 is hydrogen, methyl, ethyl, isopropyl or n-butyl.

4. A compound according to any one of claims 1 to 3 in which R_2 is n-butyl, iso-butyl or sec-butyl.

5. A compound according to any one of claims 1 to 4 in which R_3 is $-(CH_2)_nNR_5R_6$ where R_5 and R_6 are hydrogen or methyl, $-(CH_2)_nNHCOR_7$ where R_7 is $-(CH_2)_mNR_5R_6$ in which m is 1 and R_5 and R_6 are hydrogen, $-(CH_2)_nCONH(CH_2)_qNR_5R_6$ where q is 2 and R_5 and R_6 together with the nitrogen atom to which they are bonded form a 5-, 6- or 7-membered ring, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ where R_5, R_6, R_8 and R_9 are all hydrogen, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ where R_5 and R_9 together with the nitrogen atoms to which they are bonded form an optionally substituted 2-imidazolinyl group, $-(CH_2)_nR_{10}$ where R_{10} is optionally substituted piperidyl, and n is an integer from 1 to 4.

6. A compound according to any one of claims 1 to 5 in which R_4 is methyl, ethyl, $-(CH_2)_2OCH_3$, $-CH(CH_3)CO_2CH_3$ and $-(CH_2)_2OH$.

7. A compound according to any one of claims 1 to 6 in which R is hydrogen, R_1 is methyl or ethyl, R_2 is iso-butyl, R_3 is $-(CH_2)_nNR_5R_6$ where n is 3 or 4 and R_5 and R_6 are both hydrogen or methyl, $-(CH_2)_4NHCOR_7$ where R_7 is $-CH_2NH_2$, $-CH_2CONH(CH_2)_2NR_5R_6$ where R_5 and R_6 are joined together to form a pyrrolidine ring, $-(CH_2)_nNR_8C(=NR_9)NR_5R_6$ where n is 3 or 4 and R_5, R_6, R_8 and R_9 are all hydrogen, $-(CH_2)_4NR_8C(=NR_9)NR_5R_6$ where R_5 and R_9 together with the nitrogen atoms to which they are bonded form an optionally substituted 2-imidazolinyl group and R_6 and R_8 are both hydrogen, $-(CH_2)_nNHC(=NH)NH_2$ where n is 3 or 4, and $-CH_2R_{10}$ where R_{10} is 4-piperidyl; and R_4 is methyl or $-(CH_2)_2OH$.

8. A compound according to any one of claims 1 to 7 in which the chiral centre marked with an asterisk in formula (I) has the S-configuration.

9. A compound according to claim 1 which is

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-N^{ε}-glycyl-(S)-lysine methylamide;

N-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-β-[(2-pyrrolidinoethyl)amide]-(S)-aspartic acid methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-arginine methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(R)-β-(4-piperidyl)alanine methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-β-(4-piperidyl)alanine methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-ornithine methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-lysine methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-ornithine methylamide;

N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide, hydrobromide salt;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-lysine 2-hydroxyethylamide;

N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-lysine 2-hydroxyethylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide;

N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-lysine methylamide;

N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-arginine methylamide;

N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-homoarginine methylamide, disodium salt;

N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-homoarginine methylamide, disodium salt;

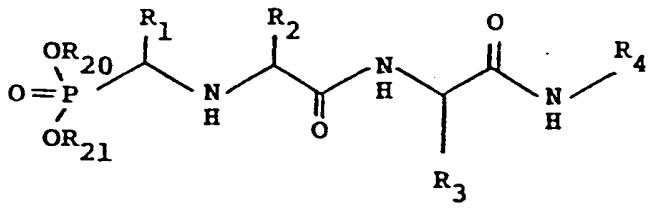
N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-(S)-N^{ε}-(2-imidazolinyl)-lysine methylamide, disodium salt;

N^{α}-[N-((S)-1-phosphonopropyl)-(S)-leucyl]-(S)-N^{ε}-(2-imidazolinyl)-lysine methylamide, disodium salt;

N^{α}-[N-((R)-1-phosphonoethyl)-(S)-leucyl]-(S)-lysine methylamide;

N^{α}-[N-((S)-1-phosphonoethyl)-(S)-leucyl]-(S)-lysine methylamide.

10. A process for the preparation of a compound as claimed in claim 1 which process comprises converting a group R_{20} to hydrogen by cleaving a group R_{20} from a compound of formula (II):



(II)

wherein R_{20} is alkyl, optionally substituted phenyl, or optionally substituted benzyl and R_{21} is hydrogen, alkyl, optionally substituted phenyl, or optionally substituted benzyl and R_1, R_2, R_3 and R_4 are as defined in formula (I), and where necessary, converting R_{21} to hydrogen, and optionally thereafter converting the compound of formula (I) to a further compound of formula (I).

11. A compound of the formula (II) as defined in claim 10 subject to the proviso that R_{21} is not hydrogen.

12. A compound according to claim 11 which is
N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-lysine methylamide;
N^{α}-[N-((R)-1-phosphonopropyl)-(S)-leucyl]-N^{ε}-(N-benzyloxycarbonylglycyl)-(S)-lysine methylamide, dibenzyl ester;
N-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-β-[(2-pyrrolidinoethyl)amide]-(S)-aspartic acid methylamide;
N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ω}-nitro-(S)-arginine methylamide;
N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(R)-β-(4-(N-benzyloxycarbonyl)piperidyl)alanine methylamide;

N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-β-(4-(N-benzyloxycarbonyl)piperidyl)alanine methylamide;
N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-benzyloxycarbonyl-(S)-ornithine methylamide;
N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-lysine methylamide;
N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ε}-dimethyl-(S)-ornithine methylamide;
N^{α}-[N-((S)-1-diethoxyphosphinylpropyl)-(S)-leucyl]-(S)-lysine methylamide;
N^{α}-[N-((R)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine 2-hydroxyethylamide;
N^{α}-[N-((S)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine 2-hydroxyethylamide;
N^{α}-[N-((S)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine methylamide;
N^{α}-[N-((S)-1-dibenzyloxyphosphinylpropyl)-(S)-leucyl]-N^{ω}-nitro-(S)-arginine methylamide;
N^{α}-[N-((R)-1-dibenzyloxyphosphinylethyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine methylamide; and
N^{α}-[N-((S)-1-dibenzyloxyphosphinylethyl)-(S)-leucyl]-(S)-N^{ε}-benzyloxycarbonyl-lysine methylamide.

13. A pharmaceutical composition comprising a compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt, solvate or hydrate thereof, and a pharmaceutically acceptable carrier.

14. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt, solvate or hydrate thereof, for use as an active therapeutic substance.

15. A compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt, solvate or hydrate thereof, for use in the treatment of conditions in which degradation of connective tissue and other proteinaceous components of the body occurs.

16. The use of a compound according to any one of claims 1 to 9 or a pharmaceutically acceptable salt, solvate or hydrate thereof, in the manufacture of a medicament for the treatment of conditions in which degradation of connective tissue and other proteinaceous components of the body occurs.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/00538

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 K 5/06, C 07 K 5/08, A 61 K 37/64, C 07 K 5/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 07 K, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	EP, A, 0320118 (BEECHAM GROUP PLC.) 14 June 1989 see page 3, line 42 - page 8, line 41; description 13, example 5; claims 1-16 cited in the application	1-16
A	Journal of Medical Chemistry, volume 33, 1990, American Chemical Society (US), Z.P. Kortylewicz et al.: "Phosphoramidate peptide inhibitors of human skin fibroblast collagenase", pages 263-273 see the whole document	1-16
A	Biochemistry, volume 28, no. 12, June 1989, American Chemical Society, (Washington, DC, US), D. Grobelny et al.: "Binding energetics of phosphorus-containing inhibitors of thermolysin", pages 4948-4951 see the whole document	1-16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th June 1991	06. 08. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	miss T. MORTENSEN

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9100538 SA 46372

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 30/07/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0320118	14-06-89	AU-A- 2507088	25-05-89
		JP-A- 1160992	23-06-89
		US-A- 4935404	19-06-90